# APPENDIX S

## Heavy Metal Concentrations in Water Potatoes in the Coeur d'Alene Basin, Idaho

# Heavy Metal Concentrations in *Sagittaria spp.* Tubers (Water Potato) in the Coeur d'Alene Basin, Idaho

FINAL DRAFT



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## Abstract

The Coeur d'Alene Basin in northern Idaho provides extensive wetland habitat, along the lower Coeur d'Alene and lower St. Joe rivers, for migrating and resident waterfowl. The lateral lakes and wetlands of the lower Coeur d'Alene River, a major portion of this habitat, have been impacted by metals-contaminated sediments related to mining activities. For years waterfowl have died in the Coeur d'Alene River wetland areas, reportedly due to lead poisoning from contaminated sediments or vegetation. *Sagittaria spp.* tubers are one of the major food sources for waterfowl in the Coeur d'Alene Basin. These tubers are also a traditional subsistence item for the Coeur d'Alene Tribe, whose aboriginal homeland is the Coeur d'Alene Basin. Tribal members gather tubers annually from the non-contaminated wetlands along the lower St. Joe River. In 1994 we sampled *Sagittaria* tubers from 14 wetlands along the lower Coeur d'Alene and St. Joe rivers to determine if tubers in Coeur d'Alene River wetlands expose waterfowl eating the tubers to metals (primarily lead), and to determine what metals concentrations tribal members would be exposed to if they were to eat tubers from Coeur d'Alene River wetlands. We also evaluated metals concentrations associated with tuber skins vs. the interior portion of the tuber. In addition, we investigated differences in mean metals concentrations in tubers between Coeur d'Alene River wetlands, and looked for relationships between tuber metals concentrations and sediment metals concentrations in wetlands from which the tubers were sampled. We found that waterfowl feeding on tubers from Coeur d'Alene River wetlands are exposed to significantly higher levels of lead, cadmium, manganese and zinc than those feeding on tubers from St. Joe River wetlands. Mean lead concentrations from Coeur d'Alene River wetlands which were

sampled ranged from 3.6 to 86.3 ppm ww, for tubers with skins. Lead was not detected in St. Joe River wetland tubers. Lead in tubers from Coeur d'Alene River wetlands exists primarily in the tuber skins, and is most likely due to sediment adhering to skins or trapped in fibrous material on the skins. If tribal members were to gather and eat tubers from Coeur d'Alene River wetlands they would likewise be exposed to significantly higher levels of lead, cadmium, manganese and zinc. Lead exposure from ingesting tubers could be greatly reduced by removing skins, however potential exposure to lead and other metals from sediment contact during the gathering process should be considered.

## Introduction

*Sagittaria latifolia* and *Sagittaria cuneata* are found in lakes and wetlands throughout the Pacific northwest United States (California, Oregon, Washington and Idaho), as well as locations on the Atlantic coast and throughout the U.S.A. (Hitchcock and Cronquist, 1973). These wetland plants produce large starchy tubers, commonly known as water potato, which serve as a major food source for waterfowl and a traditional subsistence item for the Coeur d'Alene Tribe (SiJohn, per comm.) and other native American tribes. *Sagittaria spp.* is found throughout the Coeur d'Alene Basin (CDAB), including the Coeur d'Alene (CDA) and St. Joe (STJ) River basins, of northern Idaho. This area serves as major habitat for thousands of migrating waterfowl, including ducks, Canada geese (*Branta canadensis*) and tundra swans *(Cygnus columbianus*), in the spring and to a lesser extent in the fall. Biologists have observed waterfowl feeding on *Sagittaria spp.* tubers in wetlands in the CDAB and have identified the tubers in ingesta removed from waterfowl found dead in the basin (Audet et al., 1999). The CDAB is also the home land of the Coeur d'Alene Tribe, whose members gather the tubers annually for human consumption.

The lateral lakes wetland complex is an approximately 40 km stretch of lakes and associated wetlands along the lower CDA River between the towns of Cataldo and Harrison. This area has been contaminated with heavy metals resulting from more than 100 years of mining activity upstream in the Silver Valley, a historic mining district near the town of Kellogg. Horowitz et al. (1993) estimated that 75 million tons of metal enriched sediments associated with mining activities in upstream locations of the CDARB have been deposited in the lakebed of Lake CDA. In addition, the floodplain, associated wetlands and lateral lakes of the CDA River contain large volumes of trace element-rich material, which have been released by mining activities in the upper basin and transported via the CDA River downstream to these areas. Elevated levels of hazardous substances such as lead (Pb), zinc (Zn) and cadmium (Cd), as well as other metals have been documented in the lower CDA River system (Horowitz et al., 1993; Hornig et al.,1988; Maxfield et al, 1974). Deaths of waterfowl in the lateral lakes area have been reported since 1924 (Blus et al., 1991; Neufeld, 1987; Chupp and Dalke, 1964; Chupp, 1956), many of them caused by Pb poisoning due to ingestion of Pb contaminated vegetation or sediments (Blus et al., 1991; Krieger, 1990). Elevated Pb concentrations have been found in kidney and liver tissues from waterfowl found dead in the CDA River Basin (CDARB) (Audet, 1997; Blus et al., 1991; Krieger, 1990). Neufeld (1987) suggests that the most significant cause of swan mortality in the CDARB is ingestion of Pb contaminated sediments deposited on and retained by aquatic vegetation, particularly after high water, and direct consumption of the sediments for use as grit. Lead and Zn concentrations, as well as other metals, have been reported in both submergent and emergent vegetation collected from the CDARB (Audet, 1997.; Krieger, 1990). Lead concentrations were found to be higher in equisetum samples with small quantities of CDARB sediment adhering to them than in equisetum cleaned with deionized water (Beyer et al. 1997). Audet (1997) also found Pb concentrations to be higher in sediments than in vegetation collected from the same areas.

*Sagittaria spp.* tuber contamination in the CDARB may exist as the result of sediments clinging to the tuber skins as well as from metals incorporated into the tissue of the tuber. Therefore, the tubers may serve as a pathway of exposure to heavy metals for waterfowl feeding in contaminated wetlands. According to observations of feeding behavior, the birds dig into the sediment after the tubers, potentially ingesting large amounts of sediment as well as the tubers. Beyer et al. (1998) found that waterfowl ingest as little as 2% and up to 22% sediment as diet, possibly depending on the season, the plant species and other environmental factors. Audet (1997) reported swan sediment ingestion rates in the CDAB ranging from 0 - 70%. Beyer et al. (1997) also determined that Pb exposure of wood ducks in the CDARB depended primarily on the amount of Pb- contaminated sediment ingested rather than Pb in food items.

The tubers may also present a human health concern to tribal members gathering water potatoes for consumption, as exposure may occur via ingestion of tubers with fine sediment adhering to or incorporated into skins, or from metals uptake into the pulp of the tubers. In addition tribal members may be exposed during the harvesting process, by handling sediments and inadvertently ingesting them, or by inhaling airborne particulates. The harvest of water potatoes occurs during the fall season. Water potatoes are typically collected by accessing a water potato patch (either by walking from the shore and then wading in the shallow waters/mud of the marsh, or by canoe or boat). Once the patch has been reached a digging implement is used to dig approximately 6 to 10 inches down into the sediment. Digging implements range from shovels and pitchforks to sturdy sticks. Once the sediment has been penetrated to the depth of 6 to 10 inches, the shovel full of sediment is brought to the surface. The sediment is then sorted through by hand to obtain the potatoes. Those that use sticks simply loosen the sediments with the stick and then use their hands to probe the sediment for water potatoes. Some persons wear gloves while others do not. The potatoes are then put in a sack and the process continues. Tribal members still gather water potatoes annually in wetland locations along the lower STJ River, which lies within current reservation boundaries, and has not been significantly impacted by mining activity. Although extensive *Sagittaria spp.* habitat is known to exist in the lateral lakes wetland complex along the lower CDA River, tribal members do not currently gather there because of the contamination that exists and the concern of heavy metals exposure.

***Objectives***

Due to the importance of this natural resource as both a food item to waterfowl and a cultural subsistence item to tribal members, it was determined that this investigation be designed with multiple objectives, and two different but compatible levels of sampling. Concentrations in

CDARB tubers vs. STJ River Basin (STJRB) reference area tubers, as well as metals concentrations associated with tubers collected in different wetlands in the CDARB, were evaluated to help determine which wetlands may pose a greater exposure risk to feeding waterfowl. In addition, sampling was designed to investigate the pathway of exposure, i.e., the tubers vs. the sediment in which the tubers are located. Therefore tubers from each location were analyzed both with skins (and potentially small quantities of adhering sediment) and without skins/sediment. Metals concentrations in tubers collected from individual wetlands were also compared to mean metals concentrations in sediments (Campbell et al. 1999) collected from the respective wetlands. To address human health concerns sampling was designed to simulate two different food preparation techniques used by tribal members, skinning tubers vs. eating tubers whole, to determine if removal of skins before consumption would potentially reduce exposure. In addition tubers were collected throughout the documented habitat in the CDARB and STJRB to evaluate metals concentrations associated with different potential gathering areas. Specific study objectives were as follows:

- determine if differences exist in metals concentrations in tubers between the CDARB and the STJRB;

- determine if differences exist in metals concentrations in tubers among individual wetlands in the CDARB and STJRB;

- determine if differences exist in metals concentrations between skinned and unskinned tubers from CDARB and STJRB wetlands.

- determine if a correlation exists between metals concentrations in sediments from individual wetlands in the CDARB and STJRB (Campbell et al., 1999) and metals concentrations in tubers collected from the same wetlands.

## Methods

### *Site Description*

The CDAB (Figure 1) extends from the westernmost boarder of Montana across the panhandle of northern Idaho to the Spokane River. It includes the CDARB (CDA River, tributaries, and lateral lakes wetland complex), the STJRB (STJ River and its tributaries) and Lake CDA. Major sources of metals entering the lateral lakes originate approximately 25 km upstream, from the Silver Valley mining district.

The primary *Sagittaria spp.* habitat is located in palustrine emergent areas of the lateral lakes along the CDA River, and the palustrine emergent areas of the lake system adjacent to the lower STJ River (downstream from the town of St. Maries). The lateral lakes palustrine emergent habitat encompasses approximately 5900 acres, and this stretch of STJ River palustrine emergent habitat encompasses approximately 440 acres. The habitat inventory and sample collection conducted for this investigation focused on these areas. The STJRB was selected as a reference area because of the lack of mining activity (and associated elevated metals concentrations in sediment) in the basin, the presence of similar wetland vegetation and waterfowl use, and tribal cultural concerns and use of the basin as a water potato gathering area.

### *Study Design*

In the fall of 1994, personnel from the U. S. Fish and Wildlife Service (USFWS), Coeur d'Alene Tribe and the Bureau of Land Management conducted an inventory of *Sagittaria spp.* in the CDARB and STJRB for the purpose of mapping the distribution of the vegetation. Both *Sagittaria latifolia* and *Sagittaria cuneata* were included in the inventory, since no visual differences were observed in the tubers of the two species, both exist in waterfowl feeding habitat and both are gathered as water potato by the Tribe. Locations of patches that were identified to be a minimum of 1.5 m by 1.5 m were recorded in field logs and placed on a field map. Dimensions of each patch were also noted in field logs. Patches were physically marked where practical. The inventory was conducted via ground surveys on foot or from watercraft, as well as visual surveys from vehicles. There were limitations to mapping the distribution of the plant, therefore the total area mapped was considered a conservative estimate. These limitations included:

- physical inaccessibility (i.e., some areas could not be accessed via boat, canoe, hovercraft or kayak)
- waterfowl hunting activity in wetland areas

- only easily discernable patches (no less than 2.25 square meters) were mapped
- areas requiring access to private land were not inventoried
- ability to identify the decaying vegetative component of the plant at the time of the inventory (October); only plants that could be confirmed as *Sagittaria spp.* were included in the inventory

Sampling design was based on total habitat string for each river basin, i.e., the total length of all patches documented for each basin. A target number of samples (100 in the CDARB and 50 in the STJRB) would be collected at regular intervals along each habitat string, beginning at random starting points. A total of 4540 meters of habitat string were documented in the CDARB, including patches in Harrison Slough, Thompson Marsh, Thompson Lake, Anderson Lake, Blue Lake, Cave Lake, Medicine Lake, Moffit Slough, Hidden Marsh, Campbell Marsh, Killarney Lake and Strobl Marsh. A total of 555 meters were documented in the STJRB, including patches in Lake Chatcolet, Benewah Lake and Round Lake (Figure 2). For the purposes of this study, the Plummer Creek wetland was included as part of Lake Chatcolet, since single patches of *Sagittaria spp.* extended from one wetland into the other.

Before field collection began in each basin, patches were listed from upstream to downstream, with their respective lengths, to simulate the habitat string. A collection interval was generated by dividing habitat string length by target number of samples to be collected (100 in the CDARB, 50 in the STJRB); thus the interval was 45.4 meters for the CDARB and 11.0 meters for the STJRB. The first sampling point in the first interval was randomly generated between 0 and 45.1 meters for the CDARB and between 0 and 10.7 meters for the STJRB. Random numbers were selected within a range 0.3 meters short of the length of the sampling interval to ensure that the first sampling location fell within the first interval. Subsequent sample locations were identified every 45.4 meters and 11.0 meters, respectively, from upstream to downstream along the habitat string, i.e., sampling points were assigned to patches in an upstream to downstream order. The direction of sampling along the long axis within each patch was determined by flipping a coin.

### *Field Collection*

*Sagittaria spp.* tubers were collected in November and December of 1994 from 11 of the 12 CDARB wetlands and all 3 of the STJRB wetlands (Figure 2) where *Sagittaria spp.* habitat was mapped. Anderson Lake was not sampled because only one patch was mapped there and the sampling interval was such that no sampling location fell within the patch. At each sample location within a wetland, tubers were collected along a 1 meter wide transect running perpendicular to the long axis of the patch and running the entire width of the patch (Figure 3). In general, the transect was divided into five approximately equal sections and each section excavated with a shovel until two whole tubers were located. One tuber was placed in a ziplock bag to be analyzed whole and the other placed in a ziplock bag to be analyzed with skins removed. Tubers were collected from each section until each bag contained approximately five tubers, or a total of ten tubers per sample location. If tubers were less than average size (approximately 6 cm in length, 4 cm diameter) enough were collected from each transect (a representative number from each section) to provide sufficient weight for analysis (approximately 10 grams). If tubers were difficult to locate and fewer than ten were collected from the transect, adjacent transects were excavated at one meter, two meters, etc. from the original transect on alternating sides of the original transect until ten tubers were collected. If ten tubers were not collected by the time the transect extended five meters from the original transect then the number obtained was submitted for analysis. During field collection, staff avoided collecting tubers that had come in contact with the shovel. Tubers that were damaged by the shovel, chewed on by wildlife or otherwise damaged so that sediment was embedded in the pulp of the tuber, or decayed were not collected. Each bag was labeled in the field with date, location, patch number, transect number and the collectors initials. The bags of tubers were frozen at approximately 5° F upon return from the field, and stored frozen until processed.

Prior to shipment to the laboratory all tubers were thawed and cleaned with deionized water. One portion of each sample was skinned and the other left whole, in order to simulate two different tribal food preparation techniques, as well as to provide waterfowl exposure and pathway information. The skinned tuber scenario was intended to simulate a common tribal preparation technique where tubers are peeled before cooking or canning. In addition, the skinned tuber analysis may help to better define the pathway of heavy metals exposure to waterfowl (exposure via the tuber vs. exposure via sediment adhering to the skin). The whole tuber (skins left on) scenario was intended to simulate another common tribal preparation technique where tubers are simply washed before cooking . The whole tubers would also represent the form of tuber that would be ingested by waterfowl. This scenario was considered a conservative representation of sediment ingestion by waterfowl, since birds feeding on tubers in the wild would likely ingest more sediment than was left on the prepared whole tuber samples.

Tubers to be skinned were thoroughly rinsed with deionized water and lightly scrubbed with a soft bristled brush until no sediment was visible on the surface. This was done to prevent introduction of sediment particles to the pulp of the tubers when the skin was removed. A 1 to 2 mm layer of skin was then peeled from the tubers with a plastic knife, removing all exposed surface material. The tubers were again rinsed with deionized water to remove any adhering skin or other particles. Tubers to be analyzed whole were dipped in a deionized water bath then sprayed lightly with deionized water, but not scrubbed, to remove visible sediment. Shoots and rhizomes were removed but all skin was left intact, along with any attached fibrous material. After cleaning each sample was placed in a clean, labeled bag, weighed, then frozen pending shipment to the laboratory.

A total of 145 tuber samples (95 from the CDARB and 50 from the STJRB) were submitted for analysis with skins on and 143 (93 from the CDARB and 50 from the STJRB) were submitted for analysis with skins off. One tuber sample, BL11-T9, collected from Blue Lake, was an unidentified tuber other than *Sagittaria latifolia* or *Sagittaria cuneata*. Although the plant associated with this tuber was not included in the habitat inventory, the sample was included in the metals analyses because this species of tuber has been observed in the ingesta contents of waterfowl found dead in the CDAB (J. Campbell, per observ.). This sample was smaller than the average *Sagittaria spp.* sample and therefore no skinned portion was submitted. Two other *Sagittaria spp.* tuber samples from Killarney Lake (sites KL4-T7 and KL4-T8) were inadvertently mixed during the skinning process. Therefore the skinned portions of these samples were composited and submitted as a single sample (KL4T7/8S). The unskinned portions of these samples were submitted as unique samples (KL4-T7U and KL4-T8U).

*Analytical Methods*

Tubers were shipped on dry ice to Research Triangle Institute (RTI) for metals analysis. At the laboratory samples were preserved frozen until preparation for analysis. Due to the non-homogeneous nature of the tubers, the laboratory was instructed to homogenize the entire sample in a food processor prior to subsampling. A portion of the sample was then removed, freeze dried, ground in a mill and digested in a microwave with nitric acid. Sample digestates were analyzed for six metals, including aluminum (Al), Cd, iron (Fe), manganese (Mn), Pb and Zn, by Inductively Coupled Plasma (ICP) atomic emission spectroscopy. Metals values were reported in parts per million (ppm) on a wet weight (ww) and dry weight (dw) basis. Detection limits varied from sample to sample, likely due to the variable matrix (i.e., variable amounts of fibrous material and potential sediment on the tuber exteriors). Wet weight detection limits were generally in the ranges of 2.5 - 4.0 ppm for Al, 0.04 - 0.08 ppm for Cd, 4 - 9 ppm for Fe, 0.2 - 0.3 ppm for Mn, 0.40 - 1.8 ppm for Pb and 0.45 - 0.80 ppm for Zn. Dry weight detection limits were generally in the ranges of 8 - 10 ppm for Al, 0.15 - 0.25 ppm for Cd, 15 - 23 ppm for Fe, 0.6 - 0.9 ppm for Mn, 1.5 - 4.0 ppm for Pb and 1.5 - 2.1 ppm for Zn.

*Field Quality Control Samples*

Field duplicates were collected at an approximate frequency of 6% (nine duplicate pairs for the set of 145 unskinned samples and nine duplicate pairs for the set of 143 skinned samples), to measure field variability. Since it was not practical to homogenize this matrix and split samples, field duplicates were generated as sample replicates instead of split samples, as described in USFWS and EcoChem, Inc. (1995). Duplicates were generated by collecting four tubers from each of the five transect sections, thus generating two bags for skinned analysis and two for whole analysis. Field duplicates were prepared in an identical manner to the original samples and submitted blind to the laboratory. Relative percent difference (RPD) values were calculated for metals concentrations greater than ten times the respective detection limits and compared with the 50% criteria specified in the project Quality Assurance Plan (QAP) (USFWS and EcoChem, Inc., 1995). RPD values for two of the nine unskinned duplicate pairs were above the 50% criteria for Zn concentrations. RPD values for one of the same pairs were also above criteria for Cd, Fe, and Mn concentrations. The Zn RPD value for one of the nine skinned duplicate pairs and the Mn RPD value for another skinned duplicate pair were also above the 50% criteria. Field duplicates are an indicator of variability in field sampling as well as sample matrix. Due to the replicate collection procedure, increased variance in sample measurements was expected. Since the majority of the RPD values were within the 50% criteria for all six metals, and only one duplicate pair had more than one metal outside of criteria, there was no indication of problems with field sampling relative to any specific analyte of interest (e.g., there is no indication that sampling technique is inappropriate for reproducible measurements of Pb). Therefore, there were no expected data precision problems as indicated by field duplicates. The consistently high RPD values for the one unskinned duplicate pair is more indicative of the potential for matrix variability, particularly associated with the skins of the tubers. This is likely due to the variable amounts of fibrous material found on tuber skins, which could entrap variable amounts of sediments. Since only one out of nine unskinned pairs (and no skinned pairs) demonstrated this variability, little impact on overall data quality was expected.

A sample of the deionized water used to clean the tubers prior to shipment to the laboratory was also submitted for analysis, to verify that no metals contamination occurred from the water source during sample processing. No positive result was reported by the laboratory for Al, Cd, Fe, Mn, Pb or Zn in this water sample.

***Data Validation***

Analytical data was validated for six metals of interest, including Al, Cd, Fe, Mn, Pb and Zn according to quality control (QC) criteria specified in the project QAP (USFWS and EcoChem, Inc., 1995) and to Environmental Protection Agency (EPA) guidelines for inorganic data review (USEPA, 1994). A portion of the data was subjected to 100% (full) validation in order to evaluate general data quality and to identify any analytical issues which may have affected it. The remainder of the data received limited validation which included review of any issues of concern identified in the full validation. Data validation was performed by EcoChem, Inc.

In general, all data was evaluated to be acceptable for its intended use (EcoChem, Inc., 1997a; EcoChem, Inc.,1997b) as specified in the study objectives. A number of analytical issues were reported which resulted in qualification of the data. Background concentrations of several analytes were identified in some laboratory preparation blanks, which were prepared and analyzed with the tuber samples. This indicated potential background contamination of laboratory systems. Data which may have been affected were identified and qualified as non-detected at the reported concentrations, since these concentrations may be largely attributable to laboratory contamination in addition to, or instead of, metals actually in the samples. Methods for analyzing non-detected data are discussed in statistical methods below. Additional data was qualified as estimated due to slight biases in accuracy. General high biases existed for Fe and Zn data for unskinned tubers, and for Pb data for unskinned tubers in the assessment area only, when matrix spike recoveries were evaluated.

Post-digestion spikes (analyzed to measure matrix effects and used to generate a correction factor to compensate for matrix effects) indicated a low but variable bias, particularly for unskinned tuber data. The laboratory applied a correction factor to the data to compensate for potential low recoveries indicated by the post-digestion spike data. Since post-digestion spike recoveries were as low as 53% (unskinned data) and as high as 86% (skinned data) comparisons were run on Pb data to see if variable recoveries impacted data usability, i.e., if significant differences existed between CDARB data and STJRB data, both with and without correction factors applied. An extreme-case comparison was made between CDARB unskinned data without Pb correction factors and STJRB unskinned data with Pb correction factors applied. A significant difference existed between CDARB and STJRB data, both in the extreme test and in a comparison of the corrected data sets. Therefore it was determined that post-digestion spike recoveries did not impact data usability and correction factors were left in place.

The standard reference material (SRM) associated with unskinned tubers also indicated a low bias for Pb, although the SRM matrices (citrus leaves and peach leaves) were not as representative of the sample matrix as were the matrix spike and post-digestion spike QC samples.

Finally, high variability existed in Pb data for unskinned tubers, as indicated by laboratory duplicate samples, resulting in qualification of data as estimated. This was attributed to the non-homogeneous nature of the exterior of the tuber, as some tubers had large amounts of fibrous material on the skin (which could also trap more sediment) and others did not. The laboratory reported inability to homogenize the unskinned tubers. Tubers with skins removed provided a more homogeneous matrix, and better precision data was generated with the skinned tuber data set.

Although these issues were identified in the data review process as potentially imposing some bias to the data, it was determined that none had impact on data usability. Data values were adjusted when appropriate, i.e., to compensate for laboratory background contamination and for low post-digestion spike recoveries due to matrix effects. Other QC checks (including SRM recovery outliers and laboratory duplicate outliers) warranted qualification of additional data to advise of potential bias for interpretive purposes only, but did not warrant adjusting data values. In general, laboratory difficulties in meeting some QC criteria can be attributed to the variable nature of this sample matrix. The fibrous component (and associated sediment component) of tuber exteriors varied from sample to sample, potentially contributing to variability in sample to sample measurements. In addition, this material created difficulties in sample homogenization, potentially creating within sample variability. In order to evaluate the potential impacts of this matrix on study results, we ran statistical comparisons on the most conservative data scenario and saw no differences in results. We therefore concluded that analytical issues had no impact on conclusions of this investigation. Since overall laboratory performance and data quality was considered acceptable to meet all study objectives (EcoChem, Inc., 1997a; EcoChem, Inc., 1997b) we accepted the data as reported by the laboratory for use in our analyses.

*Statistical Methods*

Statistical comparisons were run on unskinned and skinned tuber data for Al, Cd, Fe, Mn, Pb and Zn to compare :

- metals concentrations in unskinned tubers from the assessment area to those from the reference area;
- metals concentrations in unskinned tubers from each wetland to sediment data for that wetland;
- metals concentrations in skinned tubers from the assessment area to those from the reference area;
- metals concentrations in skinned tubers from each wetland to sediment data from that wetland; and

- metals concentrations in unskinned tubers to metals concentrations in skinned tubers for each wetland.

Inference from these data are to the *Sagittaria spp.* habitat identified prior to sample collection. Inferences to the identified habitat patches are made under the assumption that specimens collected are a random sample of specimens from the identified habitats. We also assume that the habitats identified prior to sampling are a representative sample from all available *Sagittaria spp.* habitats. All analyses were conducted using ww ppm values except where tuber concentrations were compared to sediment concentrations, in which case dw ppm was used for both.

Statistical comparisons of metal levels between STJRB and CDARB and between each CDARB wetland and the entire STJRB were made using Wilcoxon's two sample rank sum test (Lehmann, 1975; Hollander and Wolfe, 1973; Mann and Whitney, 1947; Wilcoxon, 1945). Wilcoxon's two sample rank sum test was performed by ranking observations from both samples, replacing any ties (note treatment of concentrations below analytical detection limits, below) with the average rank, and summing ranks over one of the samples. One-tailed significance of the rank sum was assigned using the normal approximation given by Lehmann (1975). The null hypothesis in each case was that the distribution of concentrations in the CDARB was stochastically lower or equal to the distribution of concentrations in the STJRB. The alternative hypothesis was that the CDARB distribution was stochastically higher than that of the STJRB. Reported p-values in the range 0.90 to 1.0 can be considered significant in the opposite direction (i.e., the STJRB was stochastically higher than the CDARB). All computations were carried out using S-PLUS and their testing routine wilcox.test (S-PLUS version 3.3 for Windows, © 1995 MathSoft, Inc.).

Correlation and regression analyses were conducted to investigate the relationship between mean metal (Cd, Fe, Mn, Pb and Zn) concentrations in tubers and in sediments (Campbell et al., 1999). Mean tuber and sediment concentrations were calculated from sample data within each of 14 wetlands where tubers were collected. The resulting 14 pairs of mean concentrations in tuber and sediment samples were then plotted and simple linear regression models were fit for each metal using tuber concentration as the dependent variable and sediment concentrations as the independent variable. A separate model was fit for each analyte. Fitted regression lines were plotted on the same axes. Certain metal concentration levels were reported as "below detection limit" (BDL) by the laboratory and in an effort to reduce the influence of BDL observations on the correlation analyses, calculated means were also ranked and the Spearman rank correlation coefficient was calculated for each analyte.

To analyze the effects of skinning, skinned tuber samples were paired with unskinned tuber samples collected at the same location and differences in metal concentrations were assessed using Wilcoxon's signed rank procedure (Lehmann, 1975; Hollander and Wolfe, 1973). Signed rank tests were computed for all samples combined across all wetlands in the reference and assessment areas and for each wetland from which two or more paired tuber samples were collected. There were three sites from which either the skinned or unskinned value could not be matched with a corresponding sample (BL11-T9, no skinned value; KL4-T7 and KL4-T8, no pairing because skinned tubers were inadvertently mixed). In total there were 92 paired samples from the assessment area and 50 from the reference area. Exact significance levels were computed if no (non-zero) ties existed within a pair and if there were fewer than 25 pairs, otherwise a normal approximation was used to assign significance and, in the presence of ties, the normal approximation given by Lehmann (1975) was used. All computations were carried out in S-PLUS using their routine wilcox.test with the option paired=T (S-PLUS version 3.3 for Windows, © 1995 MathSoft, Inc.). Raw paired differences were plotted to aid interpretation.

In cases where concentrations of metals were reported as BDL the laboratory reported the detection limit of the test and a qualifier specifying that the true level was below this limit. When reporting average concentrations and for graphing purposes, a value of one-half the detection limit was used in place of all BDL observations. For statistical comparisons using Wilcoxon's two sample procedure, all values (both above and below detection) in the two samples which were below the maximum detection limit of the two samples were treated as tied. For each paired test comparing metal concentrations in skinned and unskinned tubers, the largest detection limit among those values reported as BDL in both samples was computed and any value (reported as BDL or not) which was below this maximum was replaced with this maximum. For analyses correlating metal concentrations in tubers with metal concentrations in sediments (Campbell et al., 1999), concentrations reported as below analytical detection limit were replaced with a uniform random number between zero and the detection limit. Means used in the correlation analysis were then calculated including the random uniform numbers. Use of a uniform random number for values below detection accurately represents both location and variance of the resulting wetland mean, a requirement for the correlation analysis. Again, the means were also ranked and the Spearman rank correlation coefficient was calculated in order to reduce the influence of BDL observations.

## Results

### Unskinned Tubers (CDARB vs. STJRB)

Mean and median metal levels in unskinned samples from each basin appear in Table 1, along with the number and percentage of BDL values. Overall Pb, Zn, Cd, Fe and Mn levels were significantly higher in unskinned tuber samples collected in the assessment basin compared to those collected in the reference basin (all p-values <0.001, Wilcoxon test, Table 2). Aluminum levels were significantly lower in the assessment basin compared to the reference basin ($p > 0.999$, Wilcoxon test, Table 2).

Tests comparing the distribution of metals in unskinned tuber samples from assessment wetlands to the entire sample from the reference basin appear in Table 2. Lead, Zn, Cd, and Mn distributions in all assessment wetlands was significantly higher than the corresponding metal distribution in tubers from the reference area (largest p among 44 tests = 0.048, Table 2). In general, the distribution of Al was lower in the assessment wetlands when compared to the corresponding reference distribution ($p > 0.90$ for all wetlands except Thompson Marsh ($p=0.62$) and Cave Lake ($p=0.16$), Table 2). Tests involving Fe were less conclusive with Blue Lake, Campbell Marsh, Hidden Marsh, Harrison Slough, Killarney Lake, and Strobl Marsh having higher concentrations than the reference area (all p-values $< 0.054$) while the distribution of Fe in Medicine Lake was lower than in the reference area ($p = 0.945$). Box plots representing the distribution of concentrations in unskinned tubers from every wetland under study appear in Figure 4, 5, 6, 7, 8, and Figure 9. Note that a single sample was taken from Thompson Marsh, Cave Lake, and Medicine Lake in the assessment area and Benewah Lake in the reference area.

**Skinned Tubers (CDARB vs. STJRB)**

Mean and median metal levels, as well as number and percent of BDL values, for skinned tuber samples from each basin appear in Table 1. Overall Cd, Mn, and Zn levels were significantly higher in skinned tuber samples from the assessment basin (all $p < 0.001$, Wilcoxon test, Table 2). Aluminum and Fe levels were higher in skinned tubers from the reference basin (both $p > 0.999$, Wilcoxon test, Table 2). The distribution of Pb in skinned samples was marginally higher in the assessment area ($p=0.185$, Wilcoxon test, Table 2). Due to the large proportion of values below analytical detection limits, the test for Pb, to a large extent, is testing for differences between the two detected values from the assessment basin and the maximum detection limit (recall all values below the maximum detection limit were tied in this analysis).

Tests comparing the distribution of metals in skinned tuber samples from assessment wetlands to the entire sample from the reference basin appear in Table 2. Every wetland in the assessment basin contained higher levels of Cd, Mn, and Zn in skinned tubers relative to all tubers from the reference basin (largest $p = 0.055$, Table 2). Nearly every wetland in the assessment basin contained lower levels of Al and Fe in skinned tubers relative to all tubers from the reference basin (all $p > 0.90$) with the exception of Cave Lake (Al p=0.798, Fe p=0.62), Medicine Lake (Al p=0.368), Thompson Lake (Al p=0.874), and Thompson Marsh (Al p=0.798). Several relatively large readings of Al and Fe were obtained from Lake Chatcolet and Round Lake, both of which are reference wetlands (Figure 10 and Figure 12). Lead concentration distributions in skinned tubers from every assessment wetland were virtually identical to that from the reference basin with the exception of the two wetlands where values above the maximum detection limit were obtained; Blue Lake and Moffit Slough (p=0.081 and 0.028 respectively). Box plots representing the distribution of concentrations in skinned tubers from every wetland under study appear in Figure 10, 11, 12, 13, 14, and Figure 15.

**Unskinned vs. Skinned Comparisons**

Significance levels of the Wilcoxon signed rank tests comparing metal concentrations in unskinned tubers to metal concentrations in skinned tubers appear in Table 3. Overall, skinned tubers contained significantly lower concentrations of all six metals than unskinned tubers (all $p<0.001$, last row Table 3). If the same comparison is repeated on each wetland, those wetlands with five or more skinned-unskinned sample pairs showed lower concentrations of metals in skinned tubers when compared to unskinned tubers (all $p<0.062$ for all metals, except Killarney Lake Cd p=0.116, Table 3). Those wetlands where comparisons were not statistically significant also had small sample sizes (< 5 skinned-unskinned sample pairs). Lack of significant differences does not indicate that metals concentrations in skinned and unskinned samples from these wetlands are similar, but simply that there is insufficient data to determine if differences exist. In several cases, the concentration of Pb in skinned tuber material was more than 100 ppm lower than the concentration of Pb in whole tubers collected at the same location. Similarly, in several cases the amount of Zn in skinned tuber material was more than 60 ppm lower than in whole tubers collected at the same location.

**Tuber vs. Sediment**

Iron, Mn and Pb concentrations in unskinned tubers were found to be positively correlated with corresponding sediment concentrations ($p < 0.007$ Spearmans rank correlation, Table 4). Although not strongly correlated, Zn concentrations in tubers were also positively related to Zn concentrations in sediment ($p=0.31$, Table 4). Similarly, regression coefficients for Fe, Mn, and Pb calculated using parametric statistical analyses were positive ($p < 0.04$, Table 5) indicating the same positive relation between metals concentrations in tubers and corresponding sediments. A summary of the analyses for unskinned tubers can be found in Tables 4 and 5, and the data are plotted in Figure 16. Manganese, Pb and Zn concentrations in skinned tubers were positively correlated with corresponding sediment metals concentrations (Table 4 and Table 5). The strength of correlation for Mn was statistically significant at the $p=0.04$ level (Spearman's, Table 4). A summary of the analyses for skinned tubers can be found in Tables 4 and 5, and the data are plotted in Figure 17.

## Discussion

### *Waterfowl Exposure to Lead*

We found that waterfowl feeding in *Sagittaria spp.* habitat in the CDARB have greater exposure to Pb from eating the tubers than waterfowl feeding in the STJRB. This is demonstrated by the significantly higher Pb concentrations in unskinned CDARB tubers compared to unskinned STJRB tubers, for every CDARB wetland sampled. Documented sediment Pb concentrations are significantly higher in the CDARB than in the STJRB (Campbell et al., 1999). Beyer et al. (1998) determined that waterfowl in the CDAB ingest substantial quantities of sediment as part of their diet (mean sediment ingestion rates up to 22%). Tundra swan sediment ingestion rates in the CDAB have been documented as high as 70% (Audet, 1997). In addition, biologists observations have reported that birds ingest *Sagittaria spp.* tubers (with skins and adhering sediment). These findings again indicate that waterfowl feeding on *Sagittaria spp.* tubers in the CDARB have greater exposure to Pb from eating the tubers than waterfowl eating tubers in the STJRB.

Lead distributions in tubers from the CDARB were only marginally higher than in the STJRB when skins were removed. There is also an overall significant difference between Pb concentrations in skinned and unskinned tubers. This indicates that Pb is associated primarily with the tuber skins, and is not taken up by the tuber pulp to any great extent. In addition, Pb concentrations in unskinned tubers were found to be strongly correlated with sediment Pb concentrations (Campbell et al., 1999) in the wetlands from which they were collected. Lead concentrations in skinned tubers were also positively correlated with Pb concentrations in sediments from corresponding wetlands, however the strength of the correlation was weak. These findings support the conclusions of Beyer et al. (1997) that sediment is the primary source of Pb and the pathway of exposure. Tuber skins have considerable amounts of fibrous material which is in contact with the sediment while in the ground, and which could entrap various amounts of sediment, even after being rinsed. Neufeld (1987) supports this by attributing the retention of sediment on equisetum (and thus the significant increase of Pb in swan diets) to the rough surfaces of the plant. To more closely simulate what a bird would ingest, unskinned tubers analyzed for this investigation were rinsed but not scrubbed, and fibrous material was left intact, possibly along with adhering sediment. This was considered a very conservative representation of Pb exposure, since birds feeding on the tubers potentially ingest much larger quantities of sediment than were left on the study tubers.

Mean Pb concentrations for unskinned tubers collected in the CDARB ranged from 3.6 ppm ww in Cave Lake to 86.3 ppm ww in Harrison Slough. The highest tuber Pb concentration in any CDARB wetland was 126.8 ppm ww, in Moffit Slough. In comparison, (Neufeld, 1987) reported Pb in *Sagittaria spp.* tubers from the CDARB up to 400 ppm. There was, however, no indication of tuber cleaning procedures and it is possible that sediment removal from the tuber skins was not equivalent. Krieger (1990) reported Pb concentrations in tubers up to 810 ppm. Based on this report's description of sample preparation, Krieger's cleaning procedures were approximately equivalent to those used in this investigation. It is possible that the tubers with the most elevated metals concentrations were sampled from areas with the most elevated sediment metals concentrations within the CDARB wetlands. In general, mean tuber Pb concentrations from the Krieger study were elevated when mean sediment Pb concentrations from the same sites were elevated, although most mean sediment concentrations were at least 10X higher than the mean tuber concentrations from the same sites. Neufeld (1987) also reports Pb uptake by tubers, although no skinned vs. unskinned analyses was reported. As stated previously, the skinned vs. unskinned analyses performed as a part of this investigation indicate that there is no significant uptake of Pb by the tuber pulp, but that Pb concentrations are primarily associated with tuber skins and adhering sediment.

Lead concentrations in *Sagittaria spp.* tubers were evaluated as part of a Pb uptake study at various wildlife refuges in the United States (Behan et al., 1978). In this investigation *Sagittaria spp.* tubers along with other aquatic plants were collected from Pb shot areas at Squaw Creek and Ravalli National Wildlife Refuges. *Sagittaria latifolia* tuber concentrations were averaged with concentrations in river bullrush (*Scirpus fluviatilis*) and American lotus (*Nelumbo lutea*) for a mean concentration of 12.0 ppm dw. This value is comparable to the mean unskinned tuber concentration from Cave Lake (11.7 ppm dw, based on an average unskinned tuber percent moisture of 69.1%), but well below other CDARB wetland means, on a dw basis. *Sagittaria cuneata* was also collected in this study from a Pb shot seeded area and a control area. Mean concentrations for the seeded area were 7.4-7.5 ppm dw, comparable to the lowest CDARB wetland means. The control area mean concentration was <1.2 ppm dw, comparable to the STJRB unskinned tuber range of 0.94-1.1 ppm dw. Behan et al. (1978) also concluded that uptake by aquatic plants of Pb from sediments containing Pb shot or metallic Pb is not sufficient to cause Pb poisoning, and that Pb exposure of waterfowl is due primarily to the source of Pb (Pb shot or leaded sediment).

### *Waterfowl Exposure to Other Metals*

Zinc, Cd and Mn concentrations were also significantly higher in unskinned tubers from all CDARB wetlands where tubers were sampled than in unskinned tubers from the STJRB, indicating that waterfowl feeding on the tubers in the CDARB have greater exposure to these metals than waterfowl feeding in the STJRB. However, there are still significant differences in concentrations of these metals between the CDARB and STJRB when the tubers are skinned. This indicates that there is uptake of Zn, Cd and Mn to some extent by the tuber from the sediment, and that these levels do not normally exist in the tubers. It appears as though the tubers themselves serve as a pathway of exposure to Zn, Cd and Mn, as well as the sediment (as there are still overall significant differences between unskinned and skinned tubers for all of the metals).

In general, Al concentrations are higher in both unskinned and skinned tubers from the STJRB than in unskinned and skinned tubers from the CDARB. This may be due to sediment Al concentrations which have been documented as being higher in the STJRB (and in pre-mining CDARB sedimentary layers) than in sediment deposits resulting from mine tailings from the Silver Valley (Box, personal comm.). The data indicate that tubers uptake some Al, although concentrations are significantly reduced when tubers are skinned. Tests with Fe concentrations were less conclusive. Overall, Fe concentrations were found to be significantly higher in unskinned tubers collected from the CDARB than in unskinned tubers collected from the STJRB, and significantly lower in skinned CDARB tubers than in STJRB tubers.

### *Tribal Significance*

From a human consumption standpoint, these data suggest that eating tubers from wetlands in the CDARB without removing skins would present exposure to significantly higher levels of Pb and other metals than eating unskinned tubers from the STJRB.

We found that exposure to metals via ingestion of tubers collected in the CDARB could be significantly reduced by skinning the tubers. Detectable Pb levels were found in only two skinned samples from two wetlands in the CDARB, and as stated above, mean Pb concentrations from these wetlands were greatly reduced by skinning. Overall, Pb concentration distributions in the CDARB were the same as for the STJRB for skinned samples.

If cleaned reasonably well with metals-free water, no real benefit related to Pb exposure would be derived from skinning tubers collected in the STJRB, since no detectable levels of Pb were found in any tubers, skinned or unskinned, from the wetlands sampled in that basin.

It should be considered that concentrations of Pb and other metals in sediments in CDARB wetlands have been found to be significantly higher than in STJRB wetlands (Campbell et al., 1999). Exposure to Pb and other metals via sediment contact may be increased during the process of gathering tubers in the CDARB vs. gathering tubers in the STJRB, even if tubers are to be skinned prior to eating. In general, gathering tubers from wetlands in the CDARB for consumption with or without removing skins would present exposure to significantly higher levels of Zn, Cd and Mn than eating tubers from the STJRB. However, removing skins of tubers gathered in the CDARB does significantly reduce the concentrations of all three metals overall.

## Conclusions

Numerous studies have reported Pb poisoning as a cause of extensive waterfowl mortalities in the CDARB (Blus et al., 1991; Krieger, 1990; Neufeld, 1987; Chupp and Dalke, 1964). These studies suggested contaminated vegetation or sediments as the source of Pb exposure. *Sagittaria* tubers are a major food item for waterfowl in the CDARB and in the STJRB. They are also gathered by Coeur d'Alene tribal members, currently in the STJRB. This investigation was conducted to evaluate exposure of CDARB waterfowl which feed on *Sagittaria* tubers to Pb and other metals, and to evaluate metals concentrations that tribal members would be exposed to if they consumed tubers from the CDARB. We found that:

- waterfowl feeding on CDARB *Sagittaria* tubers have greater exposure to Pb, Cd, Mn and Zn than waterfowl feeding on STJRB tubers. Mean tuber Pb concentrations in the CDARB wetlands we sampled ranged from 3.6 - 86.3 ppm ww;
- lead concentrations detected in CDARB *Sagittaria* tubers are primarily associated with the skins of the tubers, and our findings suggest that sediments (probably adhering to skin

or fibrous material) are the primary source of Pb. There is some uptake of Al, Cd, Mn and Zn into the pulp of CDARB tubers, although concentrations of these metals are still higher in tubers with skins than tubers without skins;

- humans would be exposed to significantly higher levels of Pb, Cd, Mn and Zn by eating CDARB *Sagittaria* tubers with skins, as opposed to eating STJRB tubers with skins. Lead exposure from ingestion of tubers from most CDARB wetlands can be minimized to levels comparable to STJRB tubers, by removing skins prior to eating. However the potential for human exposure to Pb and other metals, via contact with sediments during the process of gathering CDARB tubers, will exists as long as sediment metals concentrations remain elevated in the CDARB.

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Figure 1. Coeur d'Alene Basin

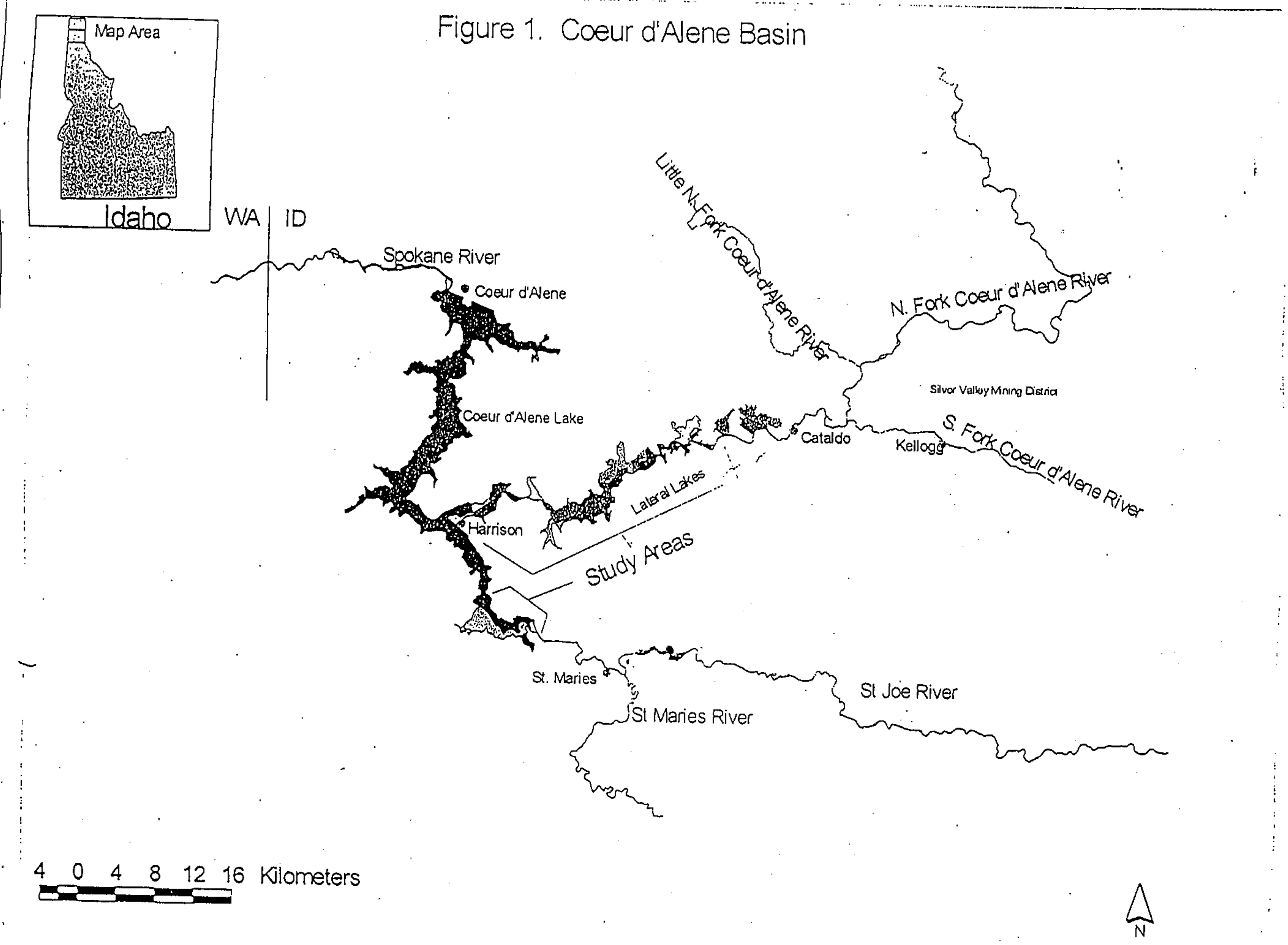


Figure 2. Wetland units included in 1994 Sagittaria habitat inventory -- Coeur d'Alene and St. Joe River Basins

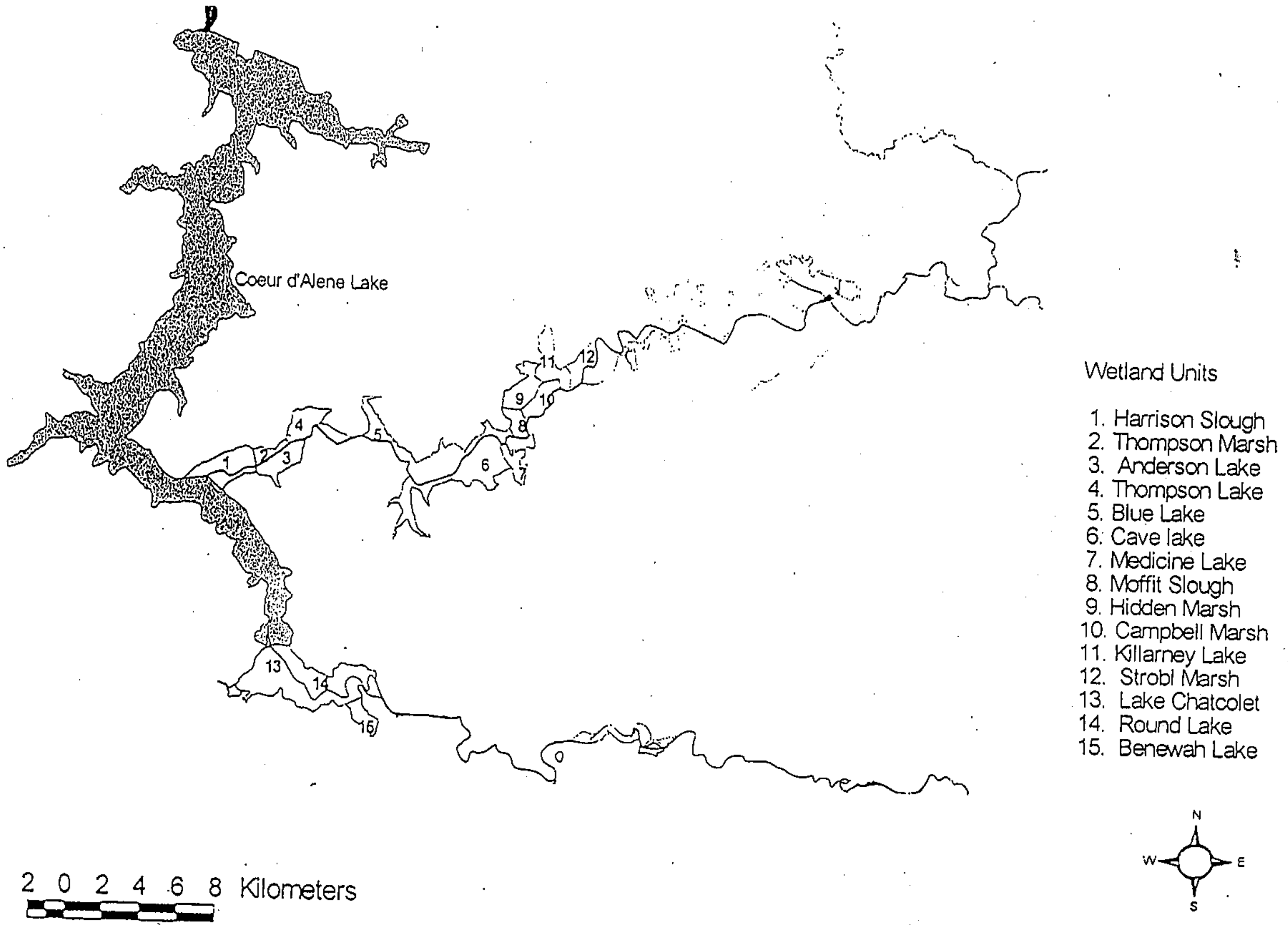
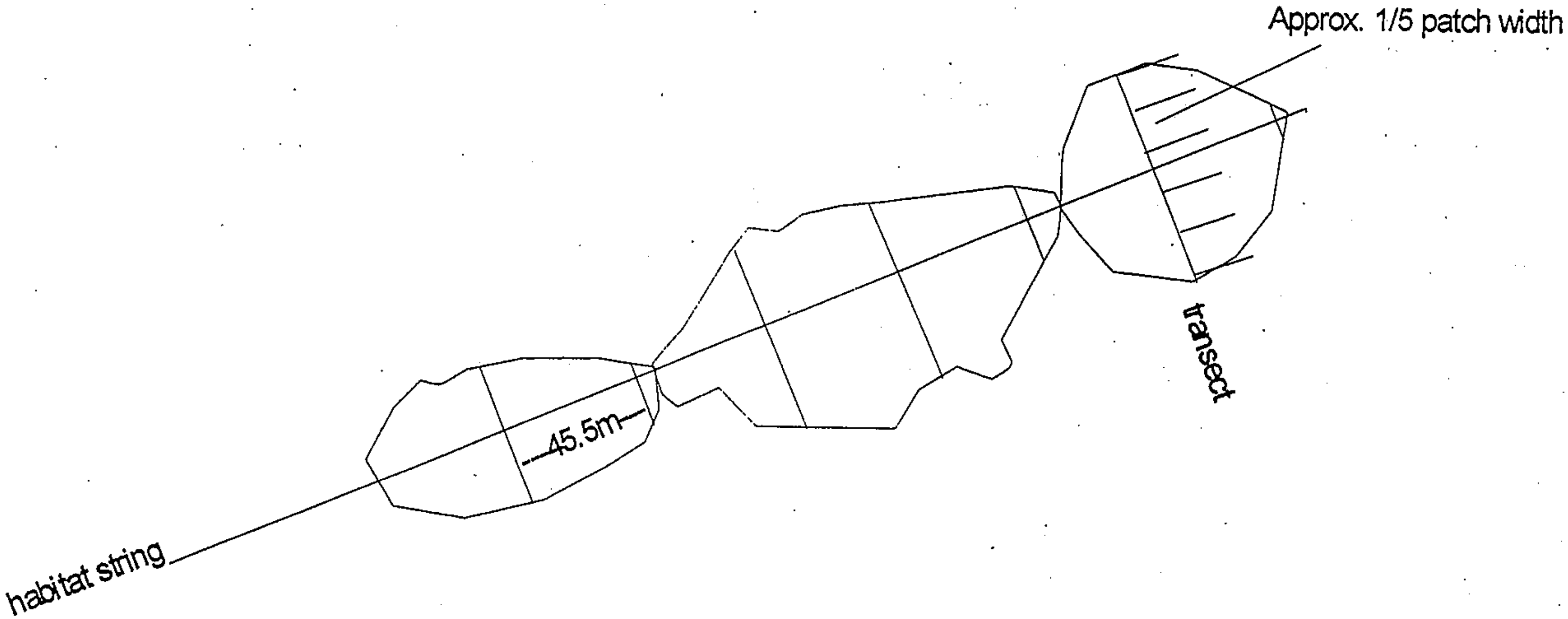


Figure 3. Sample design for tuber collection (e.g. CDARB)



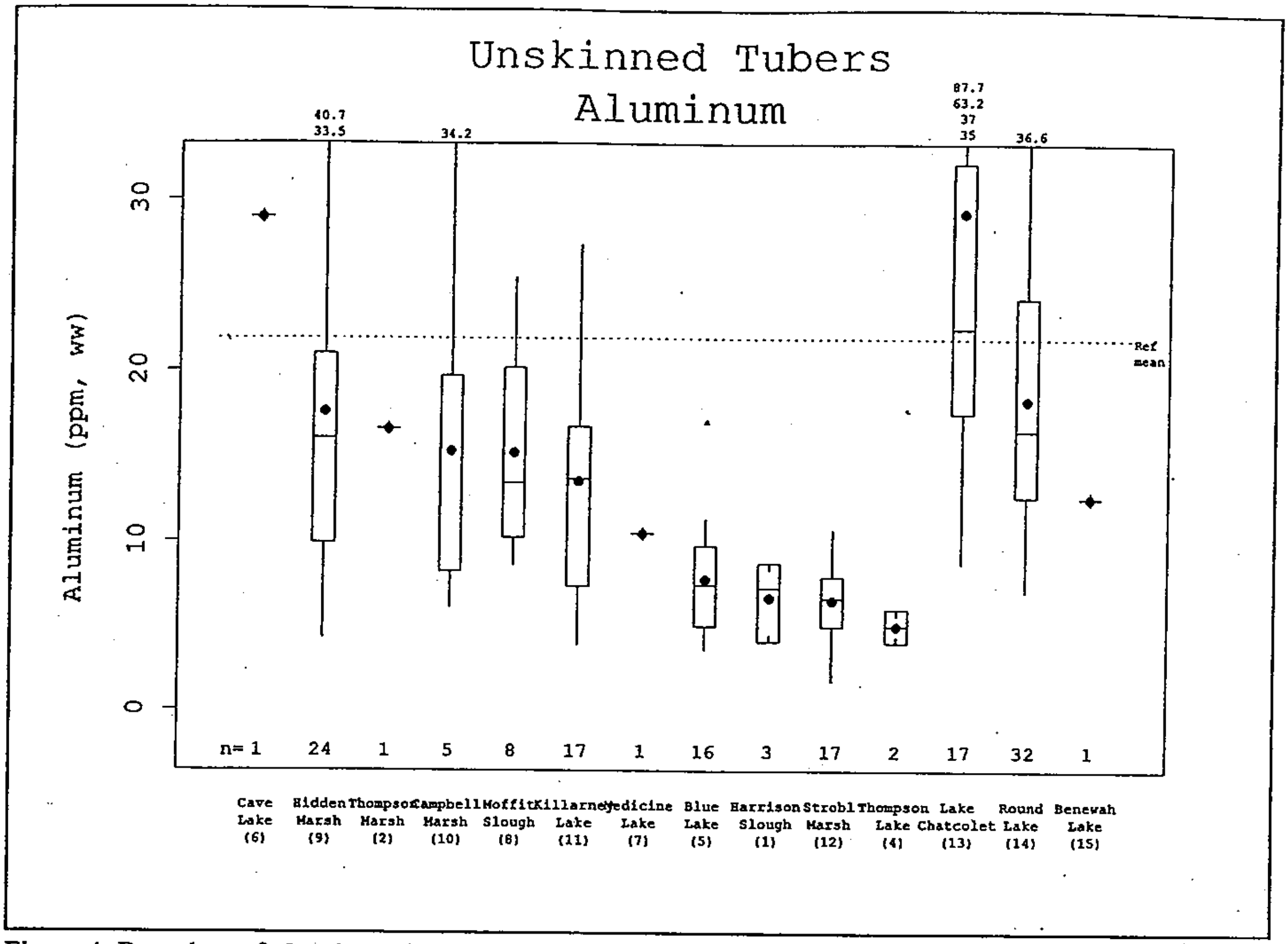



Figure 4: Box plots of **aluminum** concentrations in **unskinned** tubers for each wetland under study. Wetlands sorted by decreasing mean. Numbers at bottom inside box are sample sizes. Numbers below wetland name refer to location in Figure 2. Filled circles denote means. Box covers 25th and 75th percentiles. Line inside box denotes median. Whiskers (vertical lines) extend to observations at most 1.5 times length of box away from end of box, or to the top of the graph, which ever is smallest. Small triangles denote observations not covered by whiskers. Numbers above the graph are observed concentrations outside the range of the y-axis.

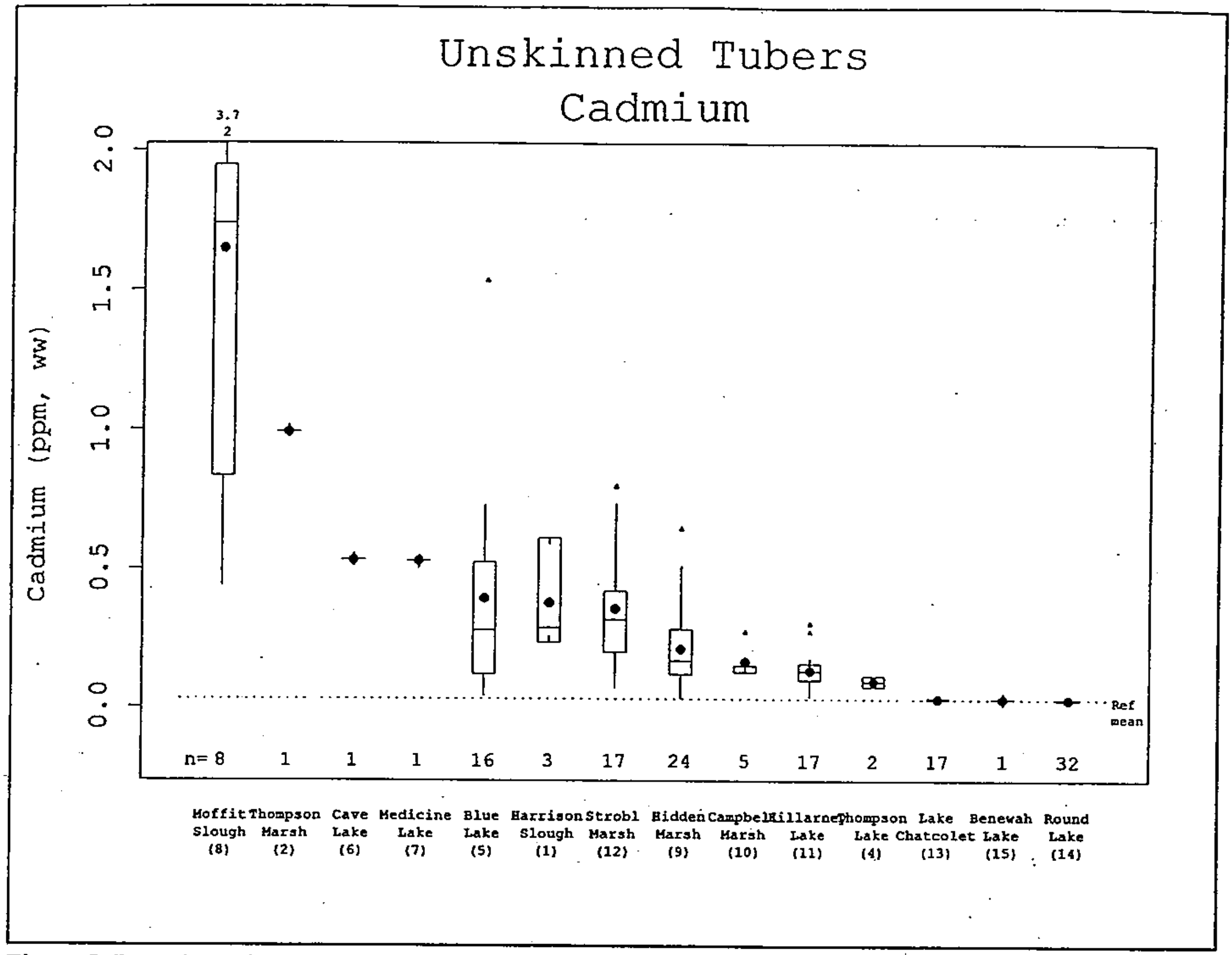



Figure 5: Box plots of **cadmium** concentrations in **unskinned** tubers for each wetland under study. Wetlands sorted by decreasing mean. Numbers at bottom inside box are sample sizes. Numbers below wetland name refer to location in Figure 2. Filled circles denote means. Box covers 25th and 75th percentiles. Line inside box denotes median. Whiskers (vertical lines) extend to observations at most 1.5 times length of box away from end of box, or to the top of the graph, which ever is smallest. Small triangles denote observations not covered by whiskers. Numbers above the graph are observed concentrations outside the range of the y-axis.

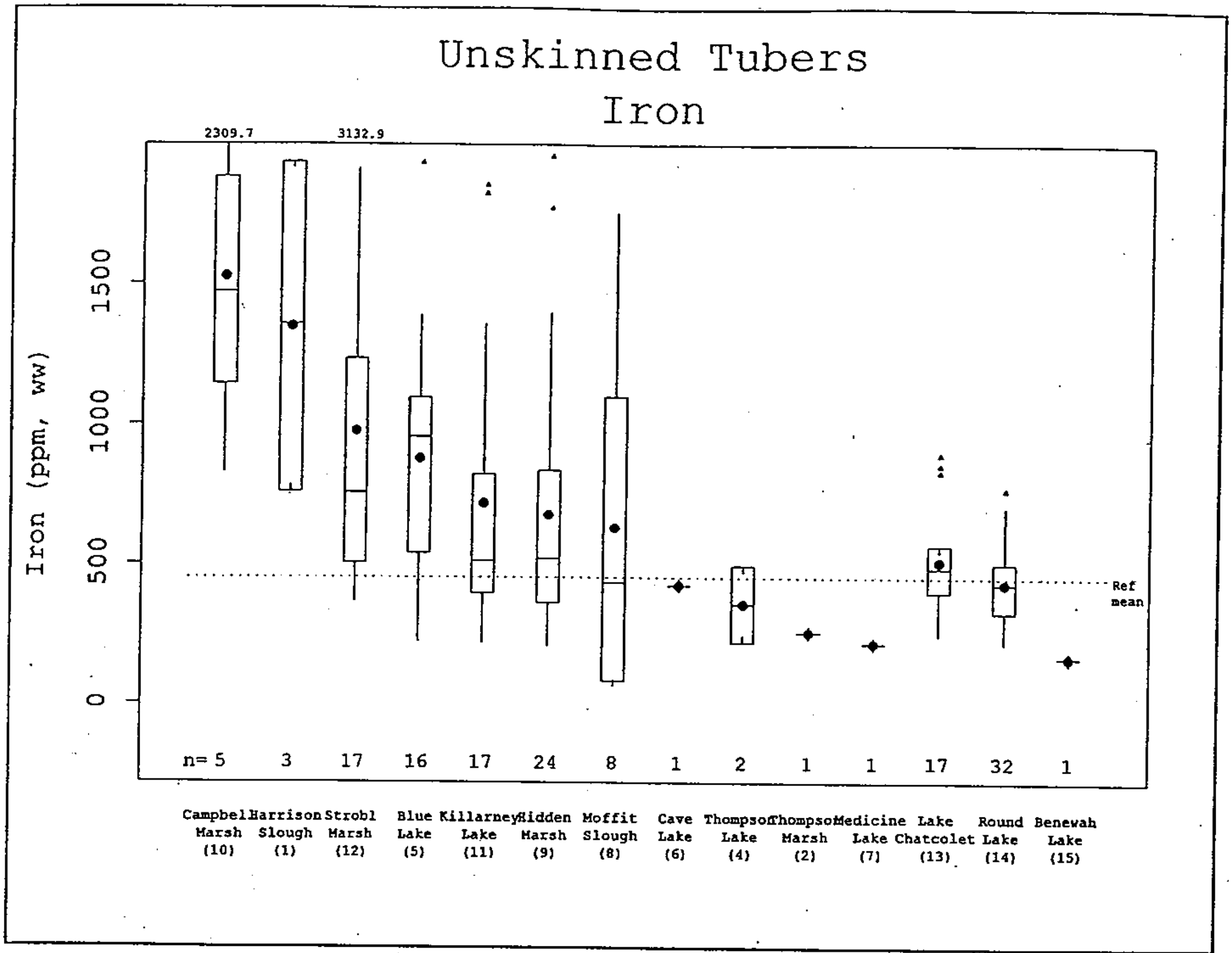



Figure 6: Box plots of **iron** concentrations in **unskinned** tubers for each wetland under study. Wetlands sorted by decreasing mean. Numbers at bottom inside box are sample sizes. Numbers below wetland name refer to location in Figure 2. Filled circles denote means. Box covers 25th and 75th percentiles. Line inside box denotes median. Whiskers (vertical lines) extend to observations at most 1.5 times length of box away from end of box, or to the top of the graph, which ever is smallest. Small triangles denote observations not covered by whiskers. Numbers above the graph are observed concentrations outside the range of the y-axis.

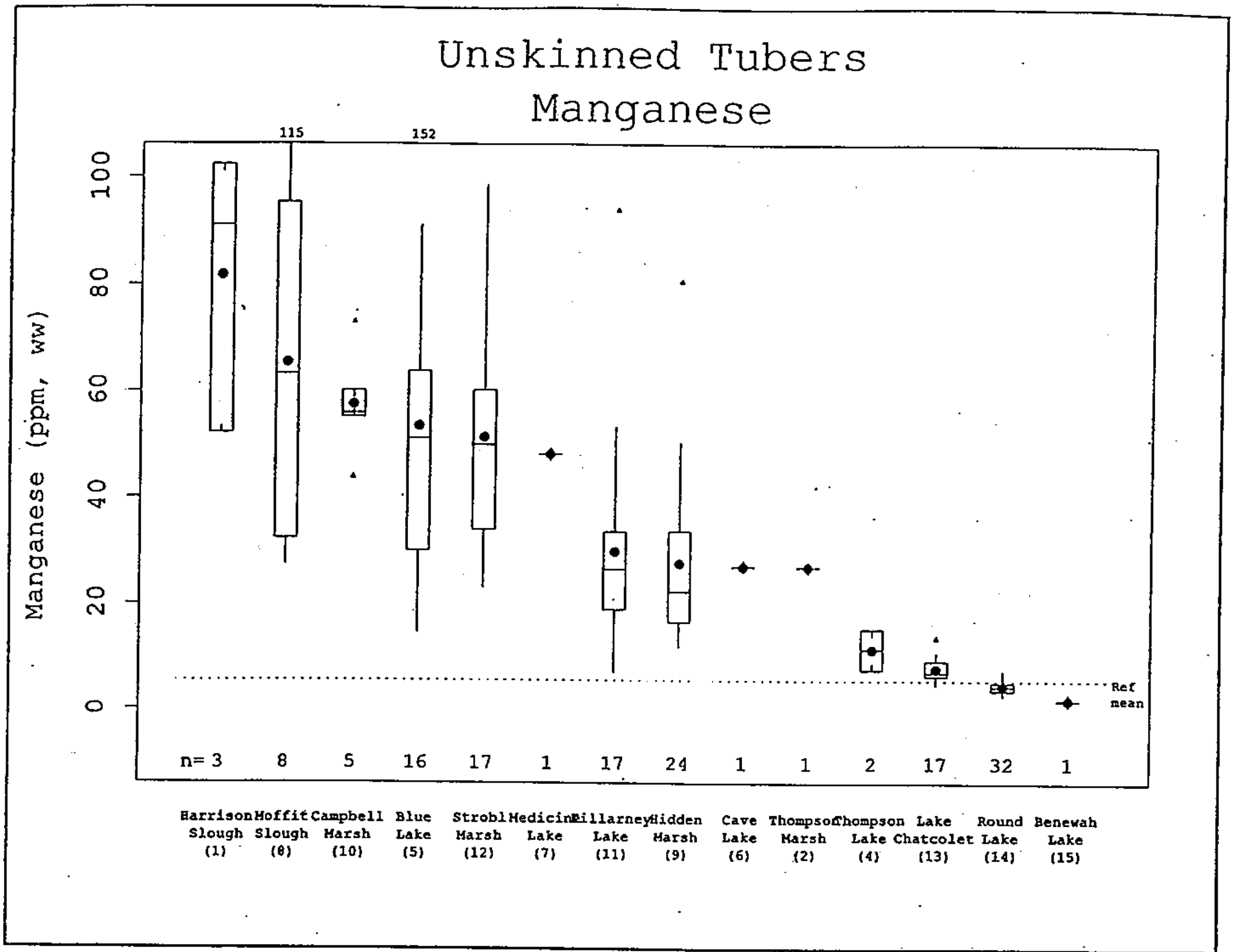



Figure 7: Box plots of **manganese** concentrations in **unskinned** tubers for each wetland under study. Wetlands sorted by decreasing mean. Numbers at bottom inside box are sample sizes. Numbers below wetland name refer to location in Figure 2. Filled circles denote means. Box covers 25th and 75th percentiles. Line inside box denotes median. Whiskers (vertical lines) extend to observations at most 1.5 times length of box away from end of box, or to the top of the graph, which ever is smallest. Small triangles denote observations not covered by whiskers. Numbers above the graph are observed concentrations outside the range of the y-axis.

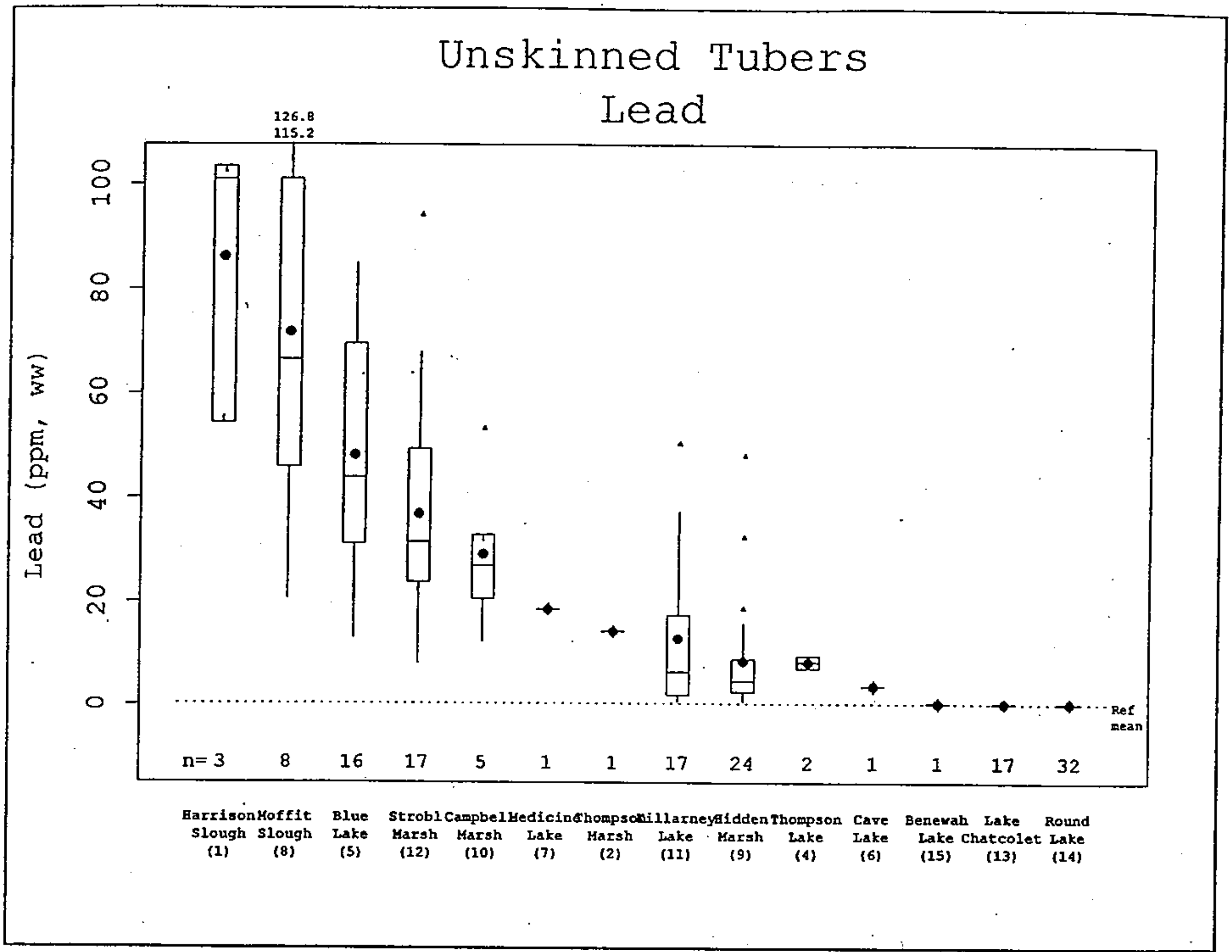



Figure 8: Box plots of **lead** concentrations in **unskinned** tubers for each wetland under study. Wetlands sorted by decreasing mean. Numbers at bottom inside box are sample sizes. Numbers below wetland name refer to location in Figure 2. Filled circles denote means. Box covers 25th and 75th percentiles. Line inside box denotes median. Whiskers (vertical lines) extend to observations at most 1.5 times length of box away from end of box, or to the top of the graph, which ever is smallest. Small triangles denote observations not covered by whiskers. Numbers above the graph are observed concentrations outside the range of the y-axis.

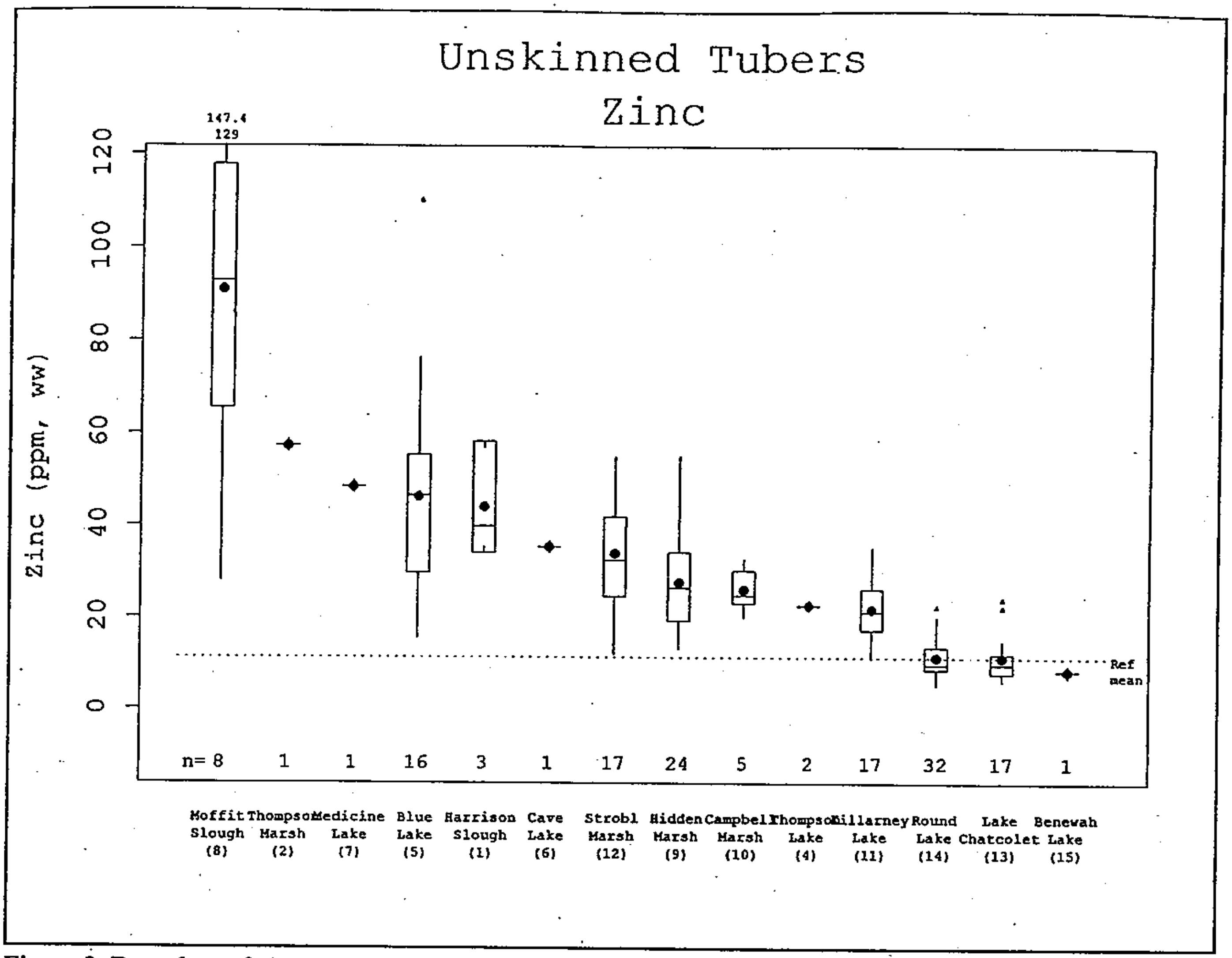



Figure 9: Box plots of **zinc** concentrations in **unskinned** tubers for each wetland under study. Wetlands sorted by decreasing mean. Numbers at bottom inside box are sample sizes. Numbers below wetland name refer to location in Figure 2. Filled circles denote means. Box covers 25th and 75th percentiles. Line inside box denotes median. Whiskers (vertical lines) extend to observations at most 1.5 times length of box away from end of box, or to the top of the graph, which ever is smallest. Small triangles denote observations not covered by whiskers. Numbers above the graph are observed concentrations outside the range of the y-axis.

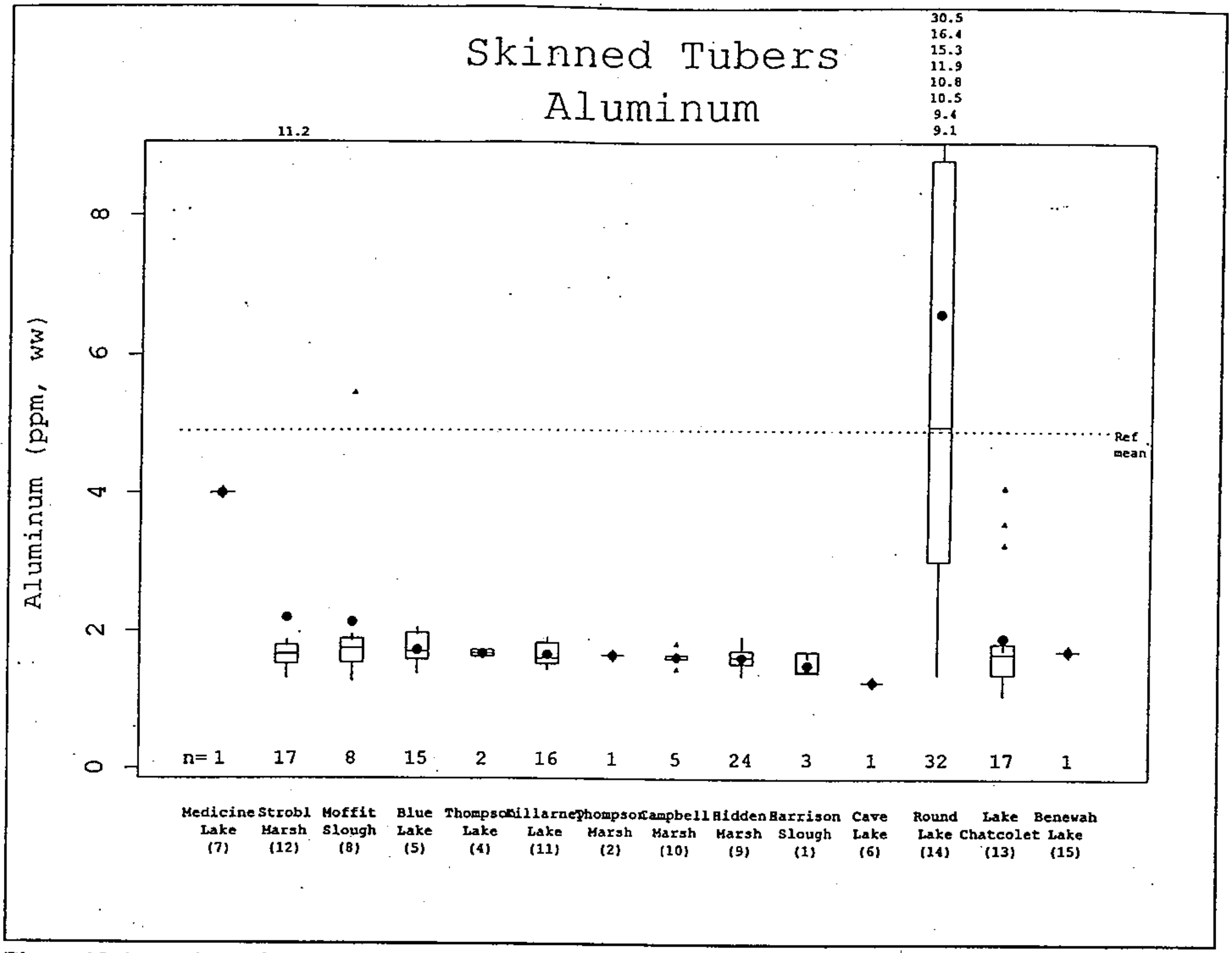



Figure 10: Box plots of **aluminum** concentrations in **skinned** tubers for each wetland under study. Wetlands sorted by decreasing mean. Numbers at bottom inside box are sample sizes. Numbers below wetland name refer to location in Figure 2. Filled circles denote means. Box covers 25th and 75th percentiles. Line inside box denotes median. Whiskers (vertical lines) extend to observations at most 1.5 times length of box away from end of box, or to the top of the graph, which ever is smallest. Small triangles denote observations not covered by whiskers. Numbers above the graph are observed concentrations outside the range of the y-axis.

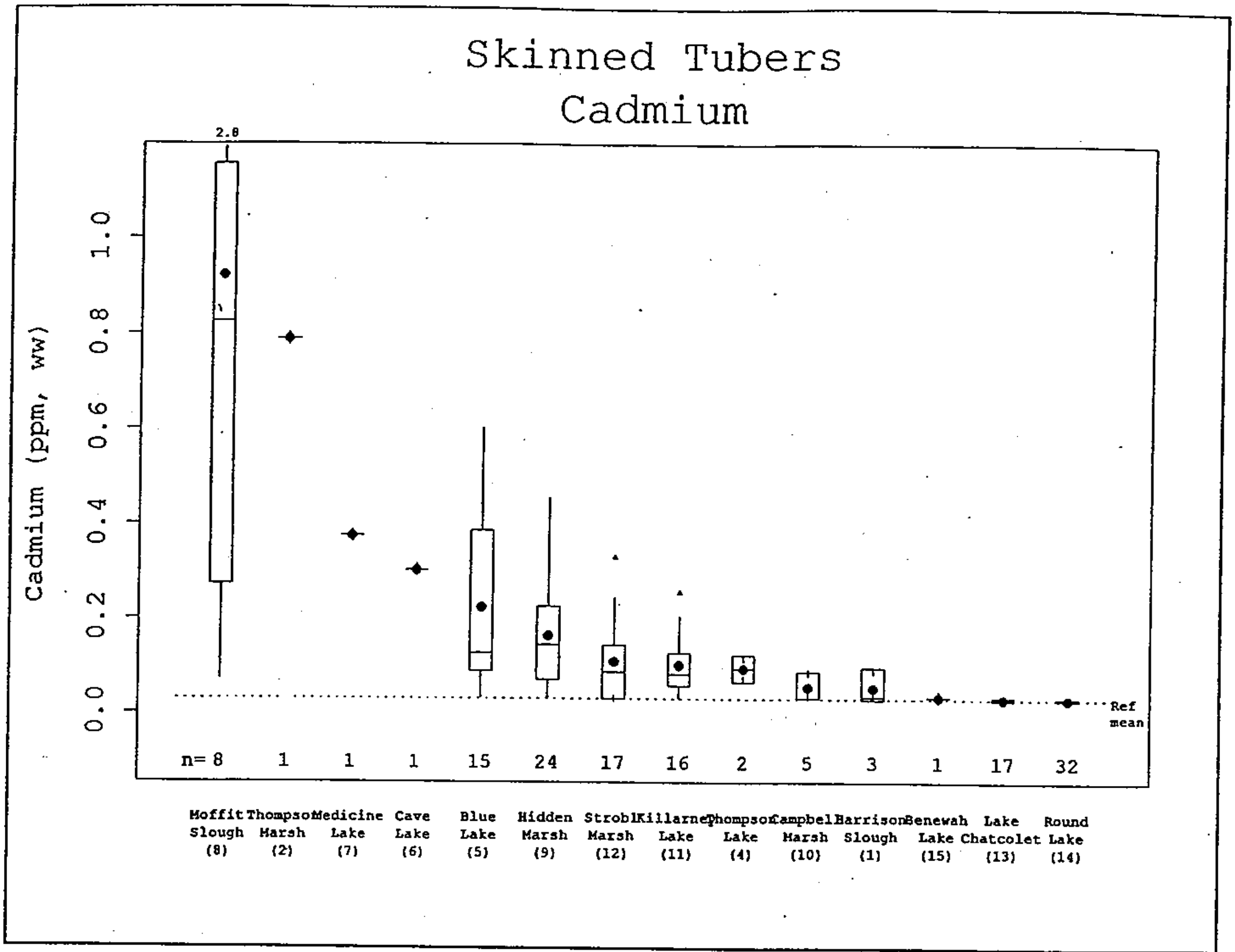



Figure 11: Box plots of **cadmium** concentrations in **skinned** tubers for each wetland under study. Wetlands sorted by decreasing mean. Numbers at bottom inside box are sample sizes. Numbers below wetland name refer to location in Figure 2. Filled circles denote means. Box covers 25th and 75th percentiles. Line inside box denotes median. Whiskers (vertical lines) extend to observations at most 1.5 times length of box away from end of box, or to the top of the graph, which ever is smallest. Small triangles denote observations not covered by whiskers. Numbers above the graph are observed concentrations outside the range of the y-axis.

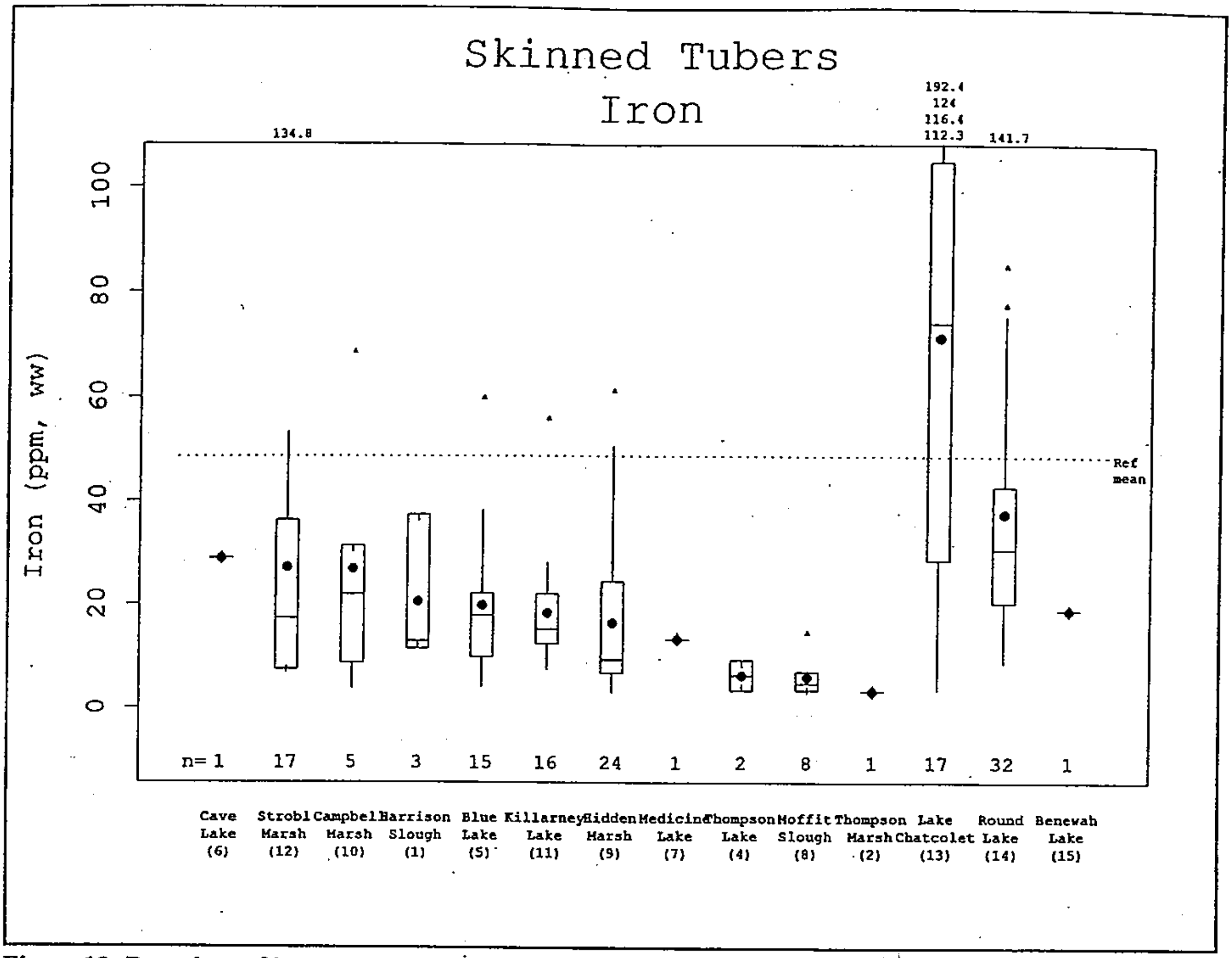



Figure 12: Box plots of **iron** concentrations in **skinned** tubers for each wetland under study. Wetlands sorted by decreasing mean. Numbers at bottom inside box are sample sizes. Numbers below wetland name refer to location in Figure 2. Filled circles denote means. Box covers 25th and 75th percentiles. Line inside box denotes median. Whiskers (vertical lines) extend to observations at most 1.5 times length of box away from end of box, or to the top of the graph, which ever is smallest. Small triangles denote observations not covered by whiskers. Numbers above the graph are observed concentrations outside the range of the y-axis.

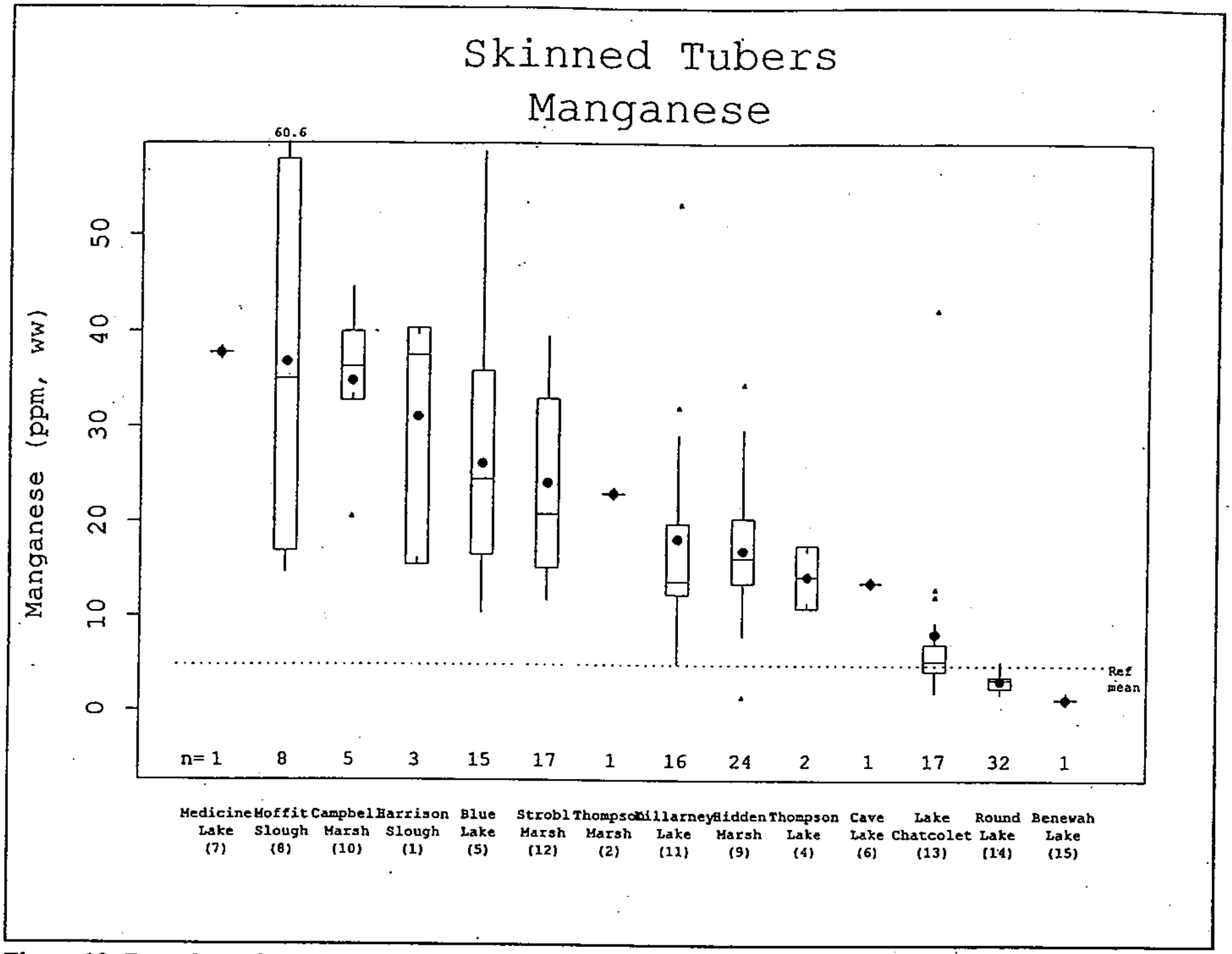



Figure 13: Box plots of **manganese** concentrations in **skinned** tubers for each wetland under study. Wetlands sorted by decreasing mean. Numbers at bottom inside box are sample sizes. Numbers below wetland name refer to location in Figure 2. Filled circles denote means. Box covers 25th and 75th percentiles. Line inside box denotes median. Whiskers (vertical lines) extend to observations at most 1.5 times length of box away from end of box, or to the top of the graph, which ever is smallest. Small triangles denote observations not covered by whiskers. Numbers above the graph are observed concentrations outside the range of the y-axis.

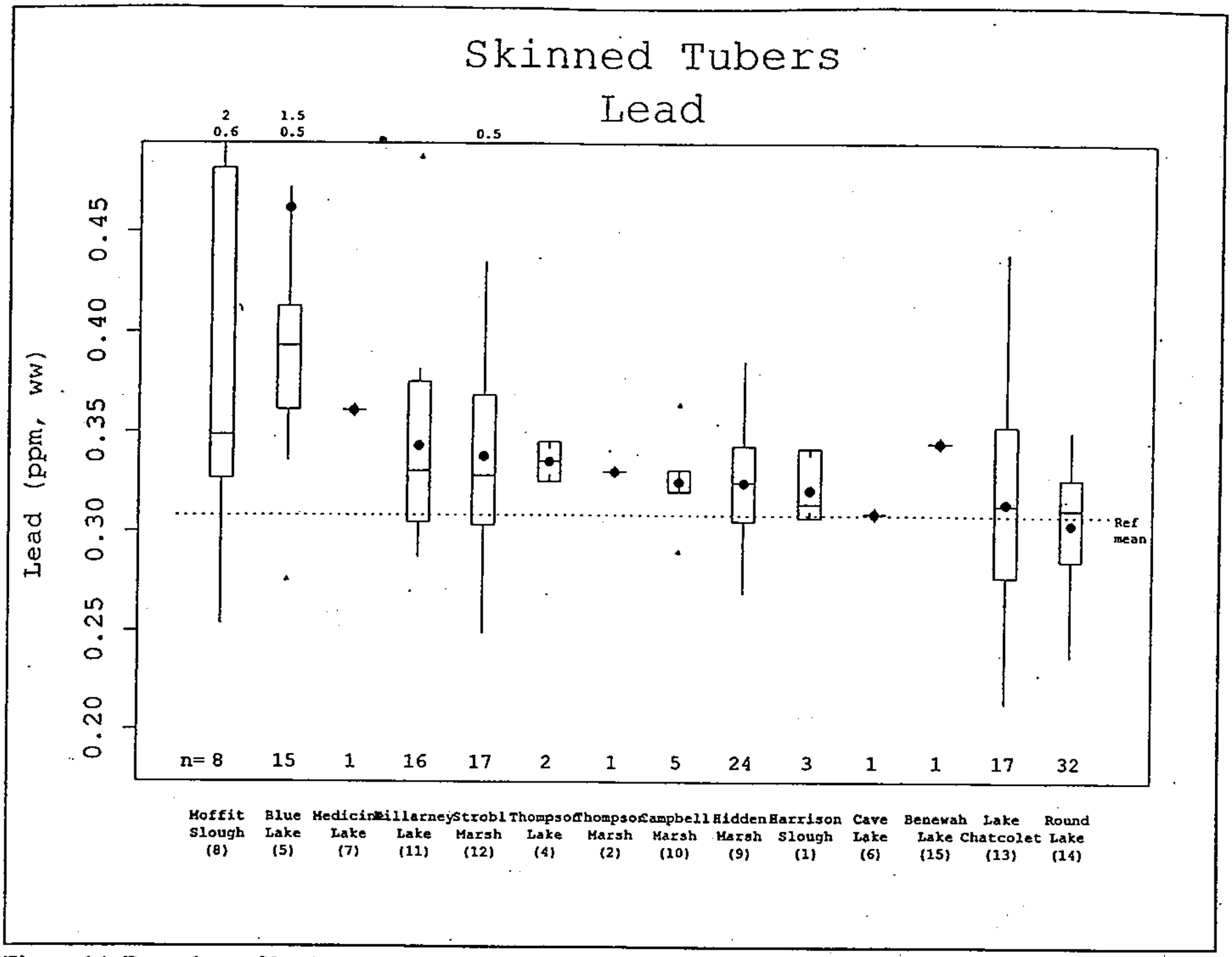



Figure 14: Box plots of **lead** concentrations in **skinned** tubers for each wetland under study. Wetlands sorted by decreasing mean. Numbers at bottom inside box are sample sizes. Numbers below wetland name refer to location in Figure 2. Filled circles denote means. Box covers 25th and 75th percentiles. Line inside box denotes median. Whiskers (vertical lines) extend to observations at most 1.5 times length of box away from end of box, or to the top of the graph, which ever is smallest. Small triangles denote observations not covered by whiskers. Numbers above the graph are observed concentrations outside the range of the y-axis.

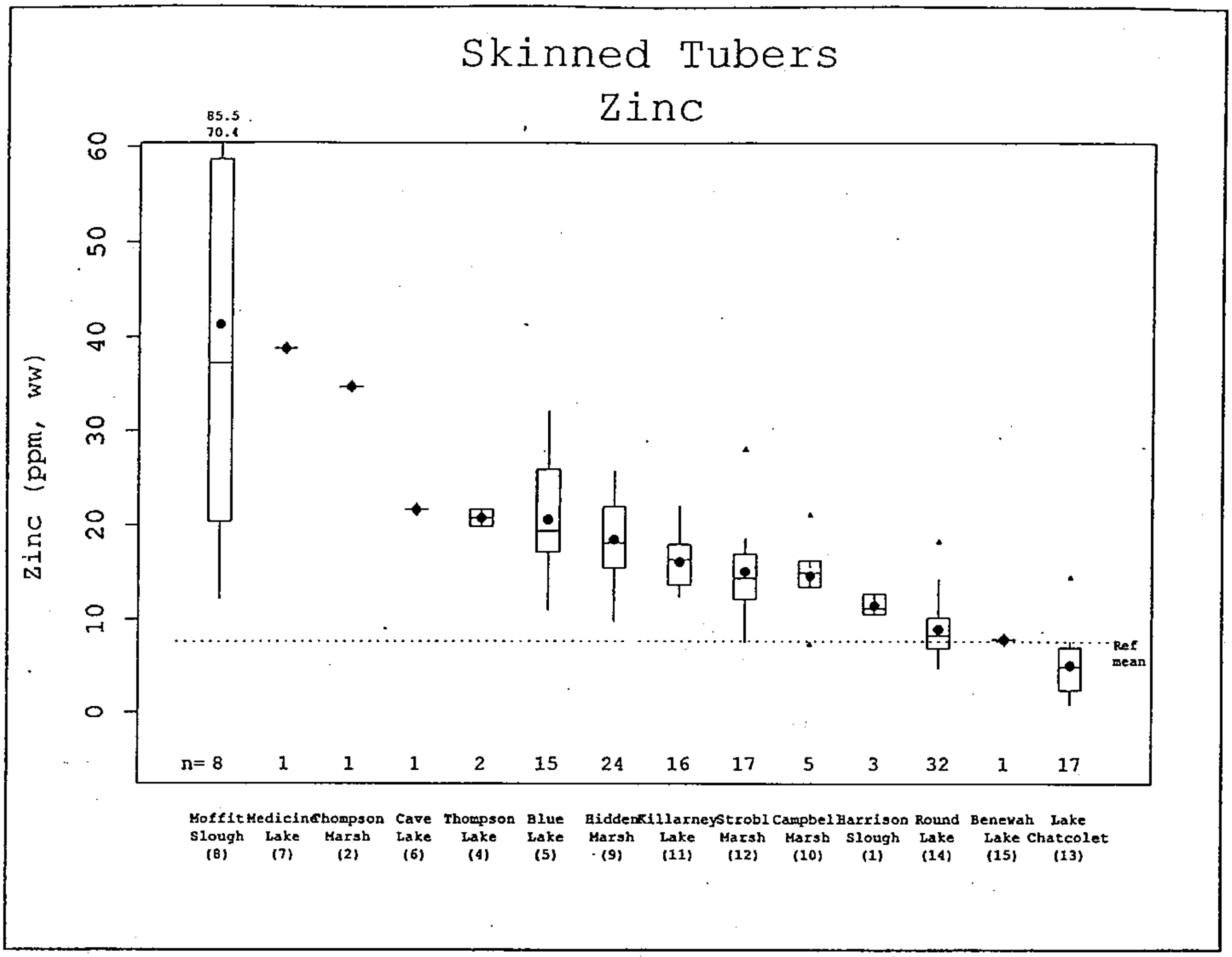



Figure 15: Box plots of **zinc** concentrations in **skinned** tubers for each wetland under study. Wetlands sorted by decreasing mean. Numbers at bottom inside box are sample sizes. Numbers below wetland name refer to location in Figure 2. Filled circles denote means. Box covers 25th and 75th percentiles. Line inside box denotes median. Whiskers (vertical lines) extend to observations at most 1.5 times length of box away from end of box, or to the top of the graph, which ever is smallest. Small triangles denote observations not covered by whiskers. Numbers above the graph are observed concentrations outside the range of the y-axis.

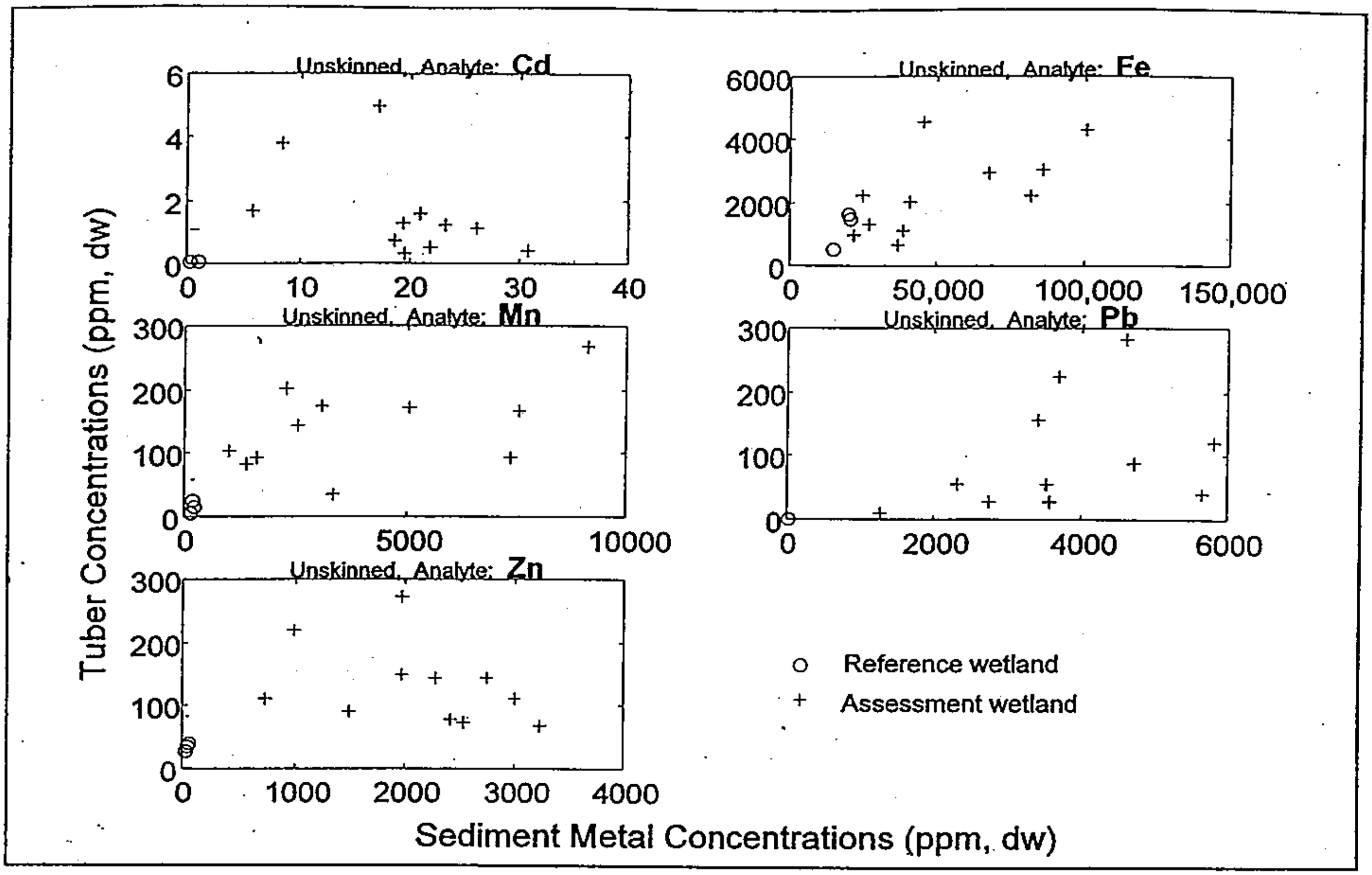



Figure 16. Metal concentrations in unskinned tubers versus metal concentrations in sediment.

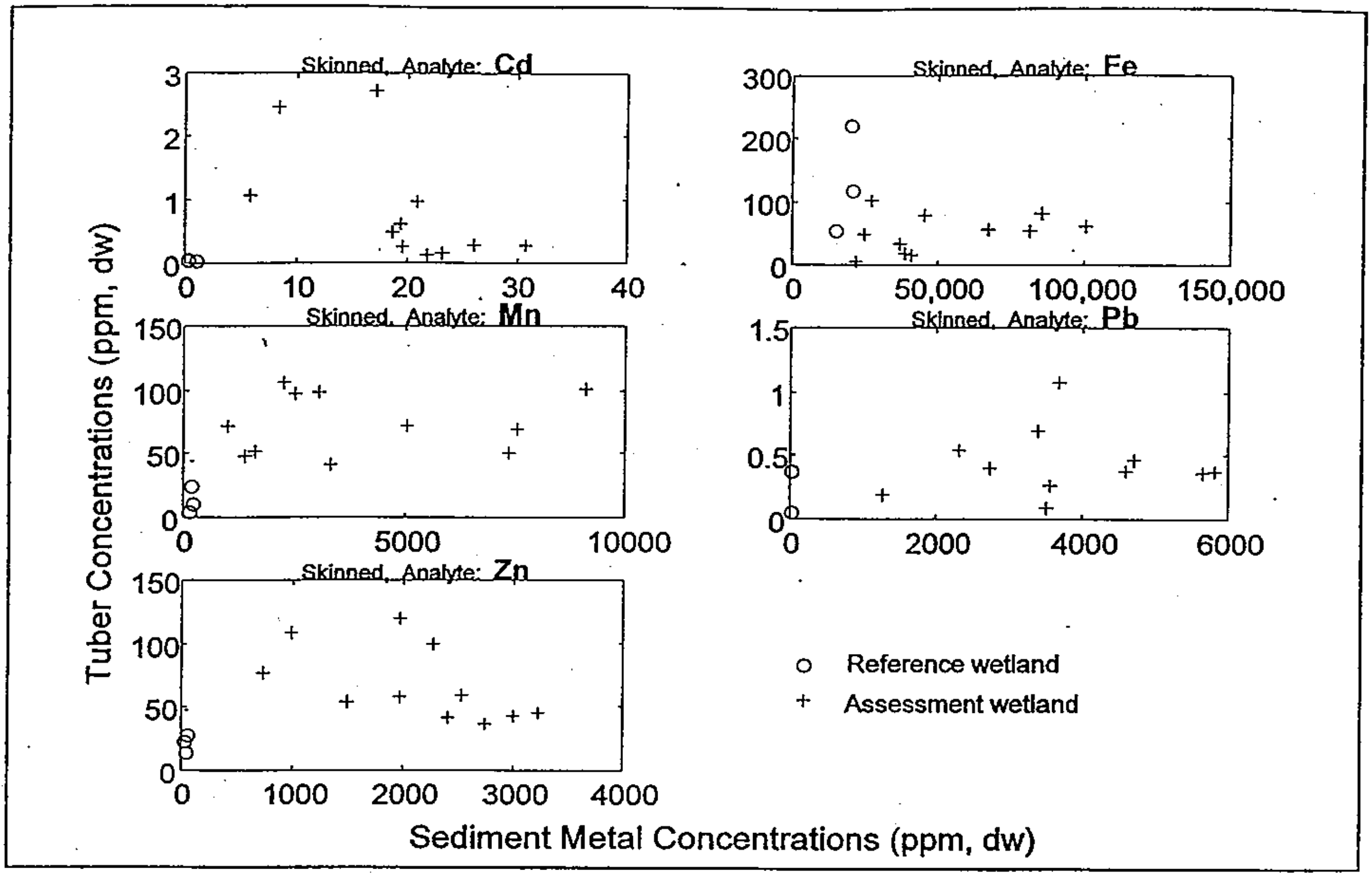



Figure 17. Metal concentrations in skinned tubers versus metal concentrations in sediment.

**Table 1**: Number of samples below detection and metal concentrations (ppm ww) in tuber samples collected in the assessment and reference basins.

| UNSKINNED TUBERS | | | | | | |
|---|---|---|---|---|---|---|
| Metal | Assessment (N= 95 ) | | | Reference (N= 50 ) | | |
| | #<LOD[a] (%) | Mean | Median | #<LOD (%) | Mean | Median |
| Aluminum | 1 (1%) | 12.3 | 9.47 | 0 (0%) | 21.89 | 18.07 |
| Cadmium | 7 (7%) | 0.39 | 0.22 | 50 (100%) | 0.03 | 0.03 |
| Iron | 0 (0%) | 813.66 | 582.47 | 0 (0%) | 451.11 | 428.77 |
| Manganese | 0 (0%) | 42.92 | 33.84 | 0 (0%) | 5.55 | 5.01 |
| Lead | 5 (5%) | 30 | 20.3 | 50 (100%) | 0.3 | 0.29 |
| Zinc | 0 (0%) | 37.08 | 29.66 | 0 (0%) | 11.28 | 9.72 |

| SKINNED TUBERS | | | | | | |
|---|---|---|---|---|---|---|
| Metal | Assessment (N= 93 ) | | | Reference (N= 50 ) | | |
| | #<LOD (%) | Mean | Median | #<LOD (%) | Mean | Median |
| Aluminum | 90 (97%) | 1.81 | 1.64 | 21 (42%) | 4.9 | 3.3 |
| Cadmium | 23 (25%) | 0.22 | 0.11 | 50 (100%) | 0.03 | 0.03 |
| Iron | 13 (14%) | 18.75 | 12.84 | 1 (2%) | 48.6 | 32.22 |
| Manganese | 0 (0%) | 23.22 | 19.03 | 0 (0%) | 4.84 | 3.43 |
| Lead | 91 (98%) | 0.37 | 0.34 | 50 (100%) | 0.31 | 0.31 |
| Zinc | 0 (0%) | 19.76 | 17.08 | 7 (14%) | 7.59 | 7.32 |

[a] LOD = Level of Detection

**Table 2:** One-sided significance levels for comparison of each assessment wetland with all reference samples based on Wilcoxon's two sample rank sum test (z test). All values below maximum detection limit were treated as tied. Small p-value implies distribution of concentrations in assessment wetland (or all) is larger than that in the reference area.

**Unskinned Tubers**

| Assessment Wetland | Metal | | | | | |
|---|---|---|---|---|---|---|
| | Aluminum | Cadmium | Iron | Manganese | Lead | Zinc |
| Blue Lake | >0.999 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Campbell Marsh | 0.93 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Cave Lake | 0.162 | <0.001 | 0.594 | 0.048 | <0.001 | 0.048 |
| Hidden Marsh | 0.901 | <0.001 | 0.025 | <0.001 | <0.001 | <0.001 |
| Harrison Slough | 0.998 | <0.001 | 0.003 | 0.002 | <0.001 | 0.002 |
| Killarney Lake | 0.997 | <0.001 | 0.054 | <0.001 | <0.001 | <0.001 |
| Moffit Slough | 0.958 | <0.001 | 0.496 | <0.001 | <0.001 | <0.001 |
| Medicine Lake | 0.918 | <0.001 | 0.945 | 0.048 | <0.001 | 0.048 |
| Strobl Marsh | >0.999 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Thompson Lake | 0.992 | <0.001 | 0.784 | 0.022 | <0.001 | 0.013 |
| Thompson Marsh | 0.62 | <0.001 | 0.883 | 0.048 | <0.001 | 0.048 |
| All | >0.999 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

**Skinned Tubers**

| Assessment Wetland | Metal | | | | | |
|---|---|---|---|---|---|---|
| | Aluminum | Cadmium | Iron | Manganese | Lead | Zinc |
| Blue Lake | 0.998 | <0.001 | >0.999 | <0.001 | 0.081 | <0.001 |
| Campbell Marsh | 0.961 | <0.001 | 0.928 | <0.001 | 0.531 | 0.003 |
| Cave Lake | 0.798 | <0.001 | 0.62 | 0.055 | 0.569 | 0.047 |
| Hidden Marsh | >0.999 | <0.001 | >0.999 | <0.001 | 0.514 | <0.001 |
| Harrison Slough | 0.917 | 0.001 | 0.908 | 0.003 | 0.54 | 0.016 |
| Killarney Lake | 0.998 | <0.001 | >0.999 | <0.001 | 0.517 | <0.001 |
| Moffit Slough | 0.936 | <0.001 | >0.999 | <0.001 | 0.028 | <0.001 |
| Medicine Lake | 0.368 | <0.001 | 0.907 | 0.055 | 0.569 | 0.047 |
| Strobl Marsh | 0.992 | <0.001 | 0.997 | <0.001 | 0.517 | <0.001 |
| Thompson Lake | 0.874 | <0.001 | 0.989 | 0.015 | 0.549 | 0.009 |
| Thompson Marsh | 0.798 | <0.001 | 0.955 | 0.055 | 0.569 | 0.047 |
| All | >0.999 | <0.001 | >0.999 | <0.001 | 0.185 | <0.001 |

**Table 3**: Two-sided significance levels comparing metal concentrations in **skinned and unskinned** tubers collected at the same location. Significance levels assigned using Wilcoxon's signed rank procedure. All values below maximum detection limit were assigned the maximum detection limit before differencing in the signed rank procedure. Wetlands containing a single skinned-unskinned pair were excluded.

| Wetland | N Pairs | Metal | | | | | |
|---|---|---|---|---|---|---|---|
| | | Aluminum | Cadmium | Iron | Manganese | Lead | Zinc |
| Blue Lake | 15 | 0.001 | 0.017 | <0.001 | <0.001 | <0.001 | <0.001 |
| Campbell Marsh | 5 | 0.062 | 0.062 | 0.062 | 0.062 | 0.062 | 0.062 |
| Hidden Marsh | 24 | <0.001 | 0.023 | <0.001 | <0.001 | <0.001 | <0:001 |
| Harrison Slough | 3 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Killarney Lake | 15 | <0.001 | 0.116 | <0.001 | <0.001 | 0.001 | <0.001 |
| Lake Chatcolet[B] | 17 | <0.001 | [A] | <0.001 | 0.045 | [A] | <0.001 |
| Moffit Slough | 8 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 |
| Round Lake[B] | 32 | <0.001 | [A] | <0.001 | <0.001 | [A] | 0.005 |
| Strobl Marsh | 17 | 0.006 | 0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Thompson Lake | 2 | 0.5 | 1 | 0.5 | 0.5 | 0.5 | 0.5 |
| All | 142 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

[A] All values below maximum detection limit. By implication, p=1.
[B] Wetland in the reference area.

**Table 4:** Spearman's rank correlation coefficient and statistical significance between wetland average metals concentration in tubers and wetland average sediment concentrations for N=14 wetlands in the reference and assessment areas.

| Analyte | Unskinned Tubers | | Skinned Tubers | |
|---|---|---|---|---|
| | Correlation Coefficient | Statistical Significance | Correlation Coefficient | Statistical Significance |
| Cd | 0.19 | 0.5126 | 0.14 | 0.63 |
| Fe | 0.71 | 0.0041 | -0.06 | 0.84 |
| Mn | 0.71 | 0.0048 | 0.54 | 0.04 |
| Pb | 0.74 | 0.0070 | 0312 | 0.29 |
| Zn | 0.29 | 0.3100 | 0.20 | 0.50 |

**Table 5:** Regression coefficient and statistical significance using parametric statistical analysis for regression of wetland average metals concentration in tubers and wetland average metals concentration in sediment for N=14 wetlands in the reference and assessment areas.

| Analyte | Unskinned Tubers | | Skinned Tubers | |
|---|---|---|---|---|
| | Regression Coefficient | Statistical Significance | Regression Coefficient | Statistical Significance |
| Cd | 0.012 | 0.78 | -0.0025 | 0.92 |
| Fe | 0.031 | 0.01 | -0.0003 | 0.57 |
| Mn | 0.018 | 0.01 | 0.0055 | 0.08 |
| Pb | 0.025 | 0.04 | 0.00003 | 0.35 |
| Zn | 0.017 | 0.32 | 0.0059 | 0.48 |

Appendix 1. Documents used in 1994 *Sagittaria spp.* Study

| | |
|---|---|
| 1019.4004 | Water Potato Sampling Protocol for Coeur d'Alene Natural Resource Damage Assessment |
| 1019.3701 | Sample Documentation and Custody Procedures (SOP) |
| 1019.3707 | Decontamination of Equipment (SOP) |
| 1019.3727 | Sample Handling and Storage-Guidance (SOP) |
| 1019.3728 | Disposal of Waste Chemicals/Samples (SOP) |
| 1019.3734 | Map and Compass Use (SOP) |
| 1019.3746 | Federal Regulations for Shipping Biological Samples (SOP) |
| 1019.3748 | Lab Processing of *Sagittaria latifolia* Tubers (SOP) |
| 1019.3850B | Coeur d'Alene Basin NRDA Wildlife Injury and Biological Pathways Studies Quality Assurance Plan |